RUBELLA CONFERENCE

APRIL 1964

DIVISION OF BIOLOGICS STANDARDS

NATIONAL INSTITUTES OF HEALTH

STENOGRAPHIC TRANSCRIPT

DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE

PUBLIC HEALTH SERVICE

NATIONAL INSTITUTES OF HEALTH

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DIVISION OF BIOLOGICS STANDARDS CONFERENCE ON RUBELLA

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Bethesda, Maryland

30 April 1964

VOLUME II

DIVISION OF BIOLOGICS STANDARDS

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DIVISION OF BIOLOGICS STANDARDS CONFERENCE ON RUBELLA

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Assembly Room
Building 29
National Institutes of Health
Bethesda, Maryland
Thursday, April 30, 1964

The meeting was convened at 9:10 o'clock a.m., Dr. Meyer presiding.

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I N D E X

- - -

DR. MEYER: If you will please come to order, we will try to get underway.

We have two or three announcements to make before we start the scientific part of the program.

(Announcements.)

DR. MEYER: I think Dr. Murray may have announcements.

DR. MURRAY: I just want to rectify an omission in my remarks yesterday. I assumed perhaps that you all appreciated this fact but a meeting of this kind, although it is by invitation, is nevertheless an open meeting inasmuch as there are a diversity of interests represented here and there is no privileged character to the information that is passed around. Anything you say is your own responsibility.

Now, I am sure that there are things that are known that are not being said. This is the privilege of the persons involved. Our main interest here is to get as much of the general feeling of the information so that we can approach this problem of eventually working on standards and standard methods so that should any immunizing agency develop there will be no holdup in the general availability of these at a later date merely because of failure to start working on standards and methods at an early enough date.

Let me emphasize again that this is in fact in the technical sense an open meeting.

Dr. Meyer?

Rubella neutralizing antibody studies

Patients	Age distribution		
	5 mo-5 yr	6 - 12 yr	13+ yr
Congen. rub.			
Neut. AB +	11	5	3
Neut. AB -	2	1	0
Controls			
Neut. AB +	1	2	0
Neut. AB -	18	3	0
+	1	0	0

Determination of Rubella Neutralizing Antibody in simultaneous maternal, fetal, cord serums and amniotic fluid from patients without clinical rubella

Gestational Age	[1] Antibody Titer Maternal	Fetal	Amniotic Fluid
10 wks	-	30	-
11 wks	>256	180	108
11 wks	775	55	<1
12 wks	-	20	-
12 wks	512	<4	<4
12 wks	-	<4	-
12 wks	-	20	-
14 wks	-	30	-
3-4 mons	-	80	-
Birth (Cord)	156	>256	
	78	1000	
	78	500	
	75	325	
	54	150	
	51	100	
	33	78	
	<4	<4	

[1] Reciprocal of 50% Neutralizing end point.

Determination of Rubella Neutralizing Antibody
in simultaneous maternal, fetal, cord serums and amniotic fluid
from patients with clinical rubella

Gestational Age	[1] Antibody Titer		
	Maternal	Fetal	Amniotic Fluid
11 wks	214	20	1
12 wks	640	16	< 4
12 wks	640	< 4	10
12 wks	10, 240	640	160
13 wks	4, 096	21	2
16 wks	> 4, 096	-	11

Birth (Cord)	Neutralizing Index	
	> 1. 5	> 1. 5
*	> 2	> 2
*	> 1. 5	> 1. 5
*	> 2. 5	> 2. 5

[1] Reciprocal of 50% Neutralizing end point

* Rubella Syndrome present at birth

1963 Epidemic

Rubella Isolates
(Products of Conception)

Total Number of Specimens	20
Positive	6
Equivocal	1
Negative	13

% Positive = 30%

1964 Epidemic

Interfering Agents
(Products of Conception)

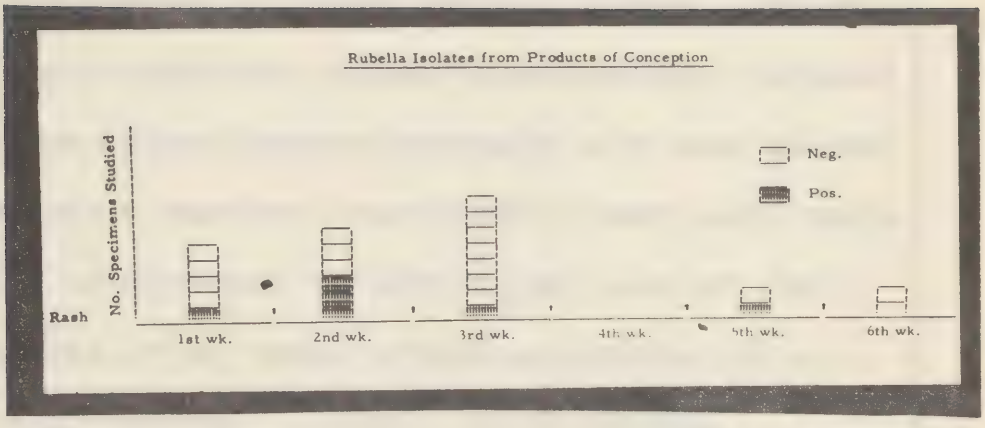
Total Number	12
Positive	7
Negative	5

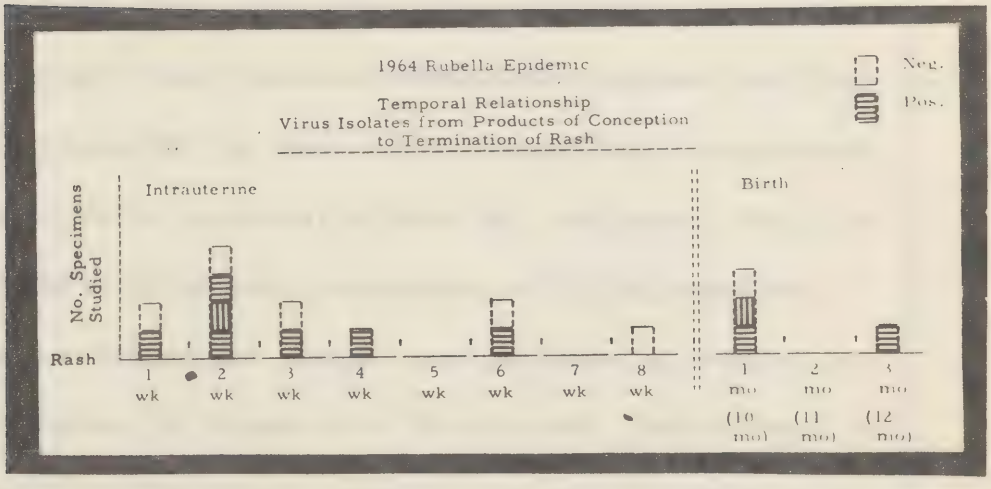
% Positive = 58%

Total Rubella Isolates
1961, 1963, 1964
(Products of Conception)

Total Number Specimens	36
Positive	13
Equivocal	1
Negative	22

% Positive = 36%


Rubella Isolates from Products of Conception
No. Specimens Studied
Neg.
Pos.
Rash
1st wk.
2nd wk.
3rd wk.
4th wk.
5th wk.
6th wk.


1964 Rubella Epidemic
Temporal Relationship
Virus Isolates from Products of Conception
to Termination of Rash
Neg.
Pos.
No. Specimens Studied
Intrauterine
Birth
Rash
1 wk
2 wk
3 wk
4 wk
5 wk
6 wk
7 wk
8 wk
1 mo (10 mo)
2 mo (11 mo)
3 mo (12 mo)

DR. MEYER: We start off this morning with a consideration of fetal infection and the rubella syndrome. There are a number of individuals who have indicated that they have information that comes under this general classification. We have not allotted time because I know at least one or two of you have nothing more than briefcase reports type of material. On the other hand, some of the others have more extensive material. I trust you can time yourself as appropriate.

I think our first speaker is Dr. Alford.

REMARKS OF DR. ALFORD

DR. ALFORD: The material to be presented today represents a composite work that has been presented over the past two years in collaboration and under the very capable guidance of Dr. Thomas Weller and Dr. Franklin Neva. Some of this work, such as the neutralizing antibody studies in sera in patients with the rubella syndrome have been completed and is now in publication. Other parts are in various stages of completion. Because of the amount of material, a detailed description of the material and methods would be impossible, so these will be given in a very general manner. Suffice to say that the techniques employed have been presented repeatedly in this meeting.

The teratogenic potential of Rubella Virus has been recognized since the initial surveys of Gregg. However, the mechanism whereby Rubella Virus exerts its deleterious effect

on the fetus have remained undefined because of the elusive nature of the virus. Since the advent of tissue cultures techniques for the isolation and maintenance of rubella virus this agent has been demonstrated in both placental and fetal tissues from an aborted human product of conception. The studies to be presented today were undertaken during recent rubella epidemics in the spring of 1963 and 1964 in the Boston area, primarily to elucidate certain of the virologic and serologic mechanisms that occur in the human fetus following maternal clinical rubella.

In 1962, prior to the rubella epidemics, sera were collected from neutralizing antibody content, patients with the stigmata of the rubella syndrome. These sera were obtained from patients under study in the cardiac, hearing and neurological clinics at Children's Hospital in Boston. Patients from the same clinic, in the same age categories, and with anomalies not considered to be due to rubella constituted the control material.

The neutralization procedure was performed on primary human amnion cell cultures employing suppression of Rubella CPE as the endpoint.

Could I have the first slide, please?

(Slide.)

In the age group from 5 months to 5 years, 11 congenital rubella sera were positive and two were negative,

whereas 1 control serum was positive and 18 negative for rubella neutralizing antibody. In the total group, 19 congenital rubella sera were positive and 3 negative, whereas 3 control sera were positive and 21 negative and 1 equivocal for rubella neutralizing antibody.

These figures agree well with those reported by Plotkin and Dudgeon from their congenital rubella series and rule out classical immunologic tolerance in most of these patients.

Further, these findings indicate that intrauterine infection with rubella virus usually induces a significant antibody response that persists for years, and that the retrospective serologic diagnosis of the rubella syndrome in the pre-school child is feasible, at least in inter-epidemic periods.

May I have the lights, please?

Further, the presence of this antibody implies the persistence of antigen until the period of immunologic competency, or that the first trimester human fetus, itself, is immunologically competent. In order to investigate this latter problem, simultaneous maternal serum, fetal serum, and amniotic fluid were obtained from patients with recent clinical rubella who had their pregnancies voluntarily interruped by hysterotomy. Usually the intact product of conception was delivered by this means. Amniotic fluid was first aspirated,

then fetal blood was obtained via the umbilical cord and diluted with ventricular fluid from the same fetus. This latter material will be referred to in this discussion as fetal serum, but it is to be emphasized that this material was initially more dilute than the simultaneous maternal serum.

Comparable specimens from hysterotomies obtained for reasons other than rubella constituted the control material. Utilizing an indirect neutralization test, employing the absence of Sindbis virus interference in rubella infected primary human amnion as the endpoint, the neutralizing antibody content of these materials was determined.

The next slide, please.

(Slide.)

It might be a bit difficult to see. The fetal sera are down to this point. Most of these represent first trimester. This is the maternal column, fetal column, and amniotic fluid. These are cord sera from this point, simultaneous maternal and fetal.

In the control serum neutralizing antibody was demonstrated in low titer in most of the fetal sera and in one amniotic fluid specimen obtained during the first trimester. The titer of this substance is very low, relative to that of the maternal serum. It seems unlikely that the initial dilution factor could account for the difference in the titer between the maternal serum and the fetal serum.

By the time of birth, however, as previously reported by Plotkin and Dudgeon, the titer of the antibody is substantial in the cord serum and, in our series, the titer in cord serum is higher per unit volume than that found in the simultaneous maternal serum.

The next slide, please.

(Slide.)

These are the titers obtained following recent maternal clinical rubella, the same quantitative distribution of neutralizing antibody was found in maternal serum, fetal serum, and amniotic fluid during the first trimester, as noted in the control group. Again the titer in the fetal serum is less than the maternal serum but greater than that of amniotic fluid. Rubella virus has been recovered from some of the fetal specimens from which these sera were derived and not from others, yet there is no quantitative difference in the distribution of the titers of the neutralizing antibody in these cases. This antibody may be found in fetal serum as early as one week following clinical rubella in the mother.

There is substantial neutralizing antibody in cord sera obtained from infants who were delivered of mothers with clinical rubella during the first trimester. In three of these infants, stigmata of the rubella syndrome were present at or shortly after birth. Interfering agents have been recovered from these infants. To date, no quantitative difference in

antibody content between the maternal serum and cord serum has been demonstrated in these cases, although the neutralization endpoints have not yet been reached. Only one of these infants appears perfectly normal and has remained so for the past five months. The level of neutralizing antibody in the cord serum of this infant is as high as in those sera from infants with rubella stigmata.

These finds indicate that active transplacental transfer of rubella neutralizing antibody does occur during pregnancy, but during the first trimester. This placental mechanism does not maintain levels of rubella neutralizing antibody in fetal serum comparable to that in the maternal serum.

Further, following infection of the mother with rubella virus and subsequent infection of the fetus, that the level of this antibody remains very low. These data do not rule out the possibility of fetal antibody production at some time during pregnancy but indicate that the physiochemical nature of the neutralizing substance will have to be determined to resolve this question.

In order to investigate the persistence of antigen during the prenatal period -- the other possibility for the neutralizing antibody in congenital rubella sera -- 95 specimens of various products of conception were obtained from 36 women whose pregnancies were voluntarily interrupted

because of recent clinical rubella. The figure is now up to 45, but incomplete. The interruptions were done by Dilatation and Curretage, Abdominal hystratomy, amenacintisis, and assisted delivery. Those specimens obtained during the 1963 rubella epidemics in the Boston area were cultured in Primary Human Amnion and African green monkey kidney.

To be accepted as a definite rubella isolate, each had to produce typical CPE and Sindbis virus interference which could be maintained on serial passage in Primary Human Amnion, typical cytopathology in stained sheets of Primary Human Amnion, interference to 1,000 $TCID_{50}$ of $ECHO_{11}$, virus on African green monkey kidney, which could be maintained on serial passage and neutralization by specific rubella rabbit antisera in African Green Monkey Kidney. Isolates obtained during the 1964 epidemic are not yet completely identified as rubella virus, according to criteria given above, and the data to be presented represents recovery of interfering agents in African Green Monkey Kidney, though most are producing typcial CPE in primary human amnion.

The next slide.

(Slide.)

This is a summary of the isolation from the 1963 epidemic. And, as you can see, rubella virus was isolated from 30 percent of the products of conception in this particular epidemic. This material, however, represented primarily

the "D" and "C" material and we cannot say for sure that this necessarily resides in either the fetus or the placenta. It could have been in the maternal tissue.

This is some pathological evidence from these tissues that there was involvement in each case, in fact in a wider percentage than we get virus isolation.

The next slide, please.

(Slide.)

The 1964 epidemic, which is still underway and still under study, the percent positive isolation is 58 percent at the present time.

The next slide.

(Slide.)

This is a composite for both epidemics. Some of these agents have not yet been identified as rubella. So far, rubella has been recovered from all the interfering material in the past and we suspect will be in these cases. There is 36 percent positive rubella isolation, we will say, in this group.

The next slide, please.

(Slide.)

This slide shows the temporal relationships of the rubella isolates from the 1963 epidemic to the termination of the rash in the mother. This line represents the termination of the rash, the number of specimens. Positive specimens are

the black and the white are negative specimens, according to our techniques that are employed. In this group, virus could be isolated from the products of conception up to 35 days following the termination of the rash in the mother.

The next slide, please.

(Slide.)

During this year's epidemic, interfering agents have been isolated from the products of conception as late as 42 days following termination of the rash in the mother. This is the last specimen up here.

Further, interfering agents with characteristics of rubella virus have been recovered from the throat and urine from three infants with stigmata of the rubella syndrome at 2 days, 10 days and 2 and a half months of age.

Lights, please.

At the present time we cannot rule out the possibility of the acquisition of rubella virus post-natally in this latter group of infants. However, one of these from whom an interfering agent has been repeatedly cultured from the throat and urine was placed in the premature nursery under strict isolation technique immediately following birth. These findings plus the presence of neutralizing antibody in patients with the rubella syndrome lend credence to the persistence of rubella virus in the fetus throughout pregnancy and after birth for an undetermined period of time. In this regard,

rubella infection of the human fetus could resemble fetal infection with cytomegalo virus.

To date, virus has been isolated from 8 placental, six fetal, and five placental-fetal admixture specimens. It has been recovered from fetal brain, heart, and skin muscle specimens. In every case when virus was isolated from fetal tissues, it has been isolated from the simultaneous placetal tissue; however, in two instances it has been isolated from placental tissue when it could not be recovered from multiple fetal specimens from the same case, which were treated in an identical manner.

I think that is enough for the present time.

(Applause.)

DR. MEYER: I am sure you will agree with me this is an extremely interesting report. I am equally sure there will be many questions.

DR. PLOTKIN: The child from whom you repeatedly recovered agents, I take it he had neutralizing antibody as well?

DR. ALFORD: Yes, they all had neutralizing antibody.

VOICE: Would you repeat that, Dr. Alford. Was that throat and urine?

DR. ALFORD: Throats and urine. We have also cultured placenta from two of those cases. The placenta were negative by the time of birth, in spite of the fact that we could recover virus from the infant itself.

DR. KRUGMAN: The blood was negative?

DR. ALFORD: The placenta. Perhaps -- we had recovered placenta isolates, for instance, in the interuterine group in every case. But in the new borns it wasn't in their placenta even though it was in the infant at that time.

DR. FELDMAN: Was that fetal antibody level constant or decreasing?

DR. ALFORD: Well, we don't know yet. These are very recent acquisitions. We intend to follow it. For the first three months it seems to be about the same.

DR. FELDMAN: Well, if you follow the fetal or

infant antibody types, after congenital infection with toxoplasma, it is about 120 days that you begin to get a very marked difference in maternal and infant antibody. But I think it would be quite crucial in these fluid antibody conditions, perhaps around four months in order to separate passive transfer antibody --

DR. ALFORD: Yes, actually we are planning to identify the physiochemical nature of whatever material is produced in this neutralization, if possible.

DR. SEVER: Have you been able to titer the virus in various organs?

DR. ALFORD: We haven't tried yet. One problem is that we were not attempting a quantitative study originally. We were just attempting viral isolates, so I am not certain that the weight to volume basis of material -- for instance when we ground material by hand that isolate virus will allow this. It is quite obvious that certain of these specimens have considerably more virus than others. For instance, the fetal brain in one case is in excess of that of the placenta, which is in excess of that of the skin muscle and so forth.

I don't know what meaning it has at the moment.

DR. GREEN: Was the diagnosis of rubella established by laboratory means in the mothers from whose fetus rubella was not isolated?

DR. ALFORD: No, and this is one reason we have not

tried to make anything from the percentages in these cases. The mothers in this series were all seen by physicians. The diagnosis was made, their histories were reviewed and oftentimes seen by three OB men at Boston Hospital before these are undertaken.

They all have neutralizing antibody. That's about all that we can say. Some of them show rises in antibody. We isolated interfering agents from the throats of a few but we were unable to recover any agent from the blood of these mothers.

DR. MEYER: That touches on a question that I had.

You did not get viremia from any of the mothers at the time of the D and C?

DR. ALFORD: No.

DR. MEYER: Did you try for viremia?

DR. ALFORD: We tried, not in every case, but we tried in a number of cases. We could never get any virus out of urine or blood so we abandoned it after we had done so many.

DR. MEYER: So insofar as you are showing that the virus at that time is not circulating actually in the fetal --

DR. ALFORD: That's right. We consider it might be in the uterine tissue and are attempting to study menses from normal females.

DR. MEYER: One other question that I think is

interesting that you just touched on, you mentioned that there were pathological findings, I assume microscopic pathological findings in many of these fetuses.

DR. ALFORD: Primarily the findings are in the placenta and this is someone else's work so I feel I shouldn't go into it into detail but there have been some pathological findings in every case from which we recover and the percentage if you take the morphological figure would be higher than those that I have presented for virus recovery.

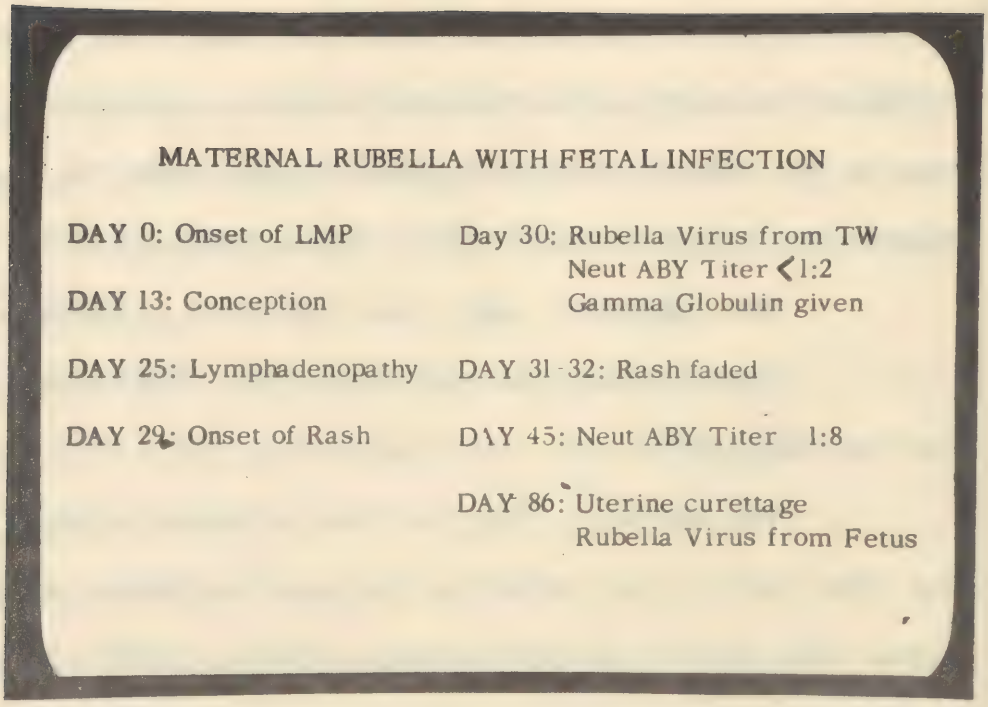
DR. KRUGMAN: May I ask one more question.

Did you have an opportunity to test blood from the new born infant?

DR. ALFORD: Yes, but one of our problems, Dr. Krugman, was that ventricular dilution factor. We have also isolated virus from brain. We did isolate it from fetal red blood cells on one occasion, but again it had been diluted with ventricular fluid.

DR. ROBBINS: Why did you dilute the blood with ventricular fluid?

DR. ALFORD: Well primarily, at the outset of this we were going to study the coagulate, the nature of the globulin in some of these sera, to determine exactly what its molecular weight was and so forth and we assumed that there is a possibility of having some globulin in ventricular fluid. Certainly there would not have been in Hank's and we had to


MATERNAL RUBELLA WITH FETAL INFECTION
DAY 0: Onset of LMP
DAY 13: Conception
DAY 25: Lymphadenopathy
DAY 29: Onset of Rash
Day 30: Rubella Virus from TW
Neut ABY Titer < 1:2
Gamma Globulin given
DAY 31-32: Rash faded
DAY 45: Neut ABY Titer 1:8
DAY 86: Uterine curettage
Rubella Virus from Fetus

have some dilution in order to carry out the procedures.

There is an insufficient quantity of serum that would be obtained to perform these tests.

DR. MEYER: Thank you Dr. Alford.

I think next we have Dr. Heggie with more of the same.

REMARKS OF DR. HEGGIE

DR. HEGGIE: I would like to report a case from which we were able to isolate rubella virus from the throat early in pregnancy and from the fetal tissue after about three months gestation when the pregnancy was interrupted. I think I can do it all from this one slide.

(Slide)

This lady had determined her basal body temperature during her previous menstral cycle, having intended to become pregnant at this time, and so we were able to plot from this chart she had maintained, the probable date of conception.

If we call today zero the onset of the last menstral period from this chart she had kept she had conceived approximately on date 13. On day 25 she was noted by her physician to have lymphadenopathy of the posterior occipital type and she had the onset of the rubella rash on the 29th day after the onset of her last menstral period.

She appeared in the emergency room of our hospital on day 30 having been sent in by this physician to receive

gamma globulin, it being one of the responsibilities of this hospital to give gamma if the physician sends the patient in requesting it.

She received at that time 20 milliliters of ordinary commercial gamma globulin and throat washing and acute serum were obtained from her. From the throat washing we were able to isolate rubella virus and her neutralizing antibody titer, this all being done in the African green monkey system.

Her neutralizing antibody titer was less than one to two.

Day 31-32 her rash faded. After her rash faded we were able to obtain a convalescent serum and her titer had gone up from one to eight.

She was lost to follow up on. We did not hear from her again until she consulted an obstetrician for pregnancy care and he after consulting with her decided to terminate the pregancy by "D and C". And from these fetal tissues which we received, we made an extract and we made some explant cultures. The tissue is not specifically identifiable except for a piece of a lower extremity, why I presume that these cultures we were able to grow were essentially skin muscle preparations. And from both the extracts made from this fetal tissue and from the supernatant fluids overlayed the explant cultures we were able to isolate rubella virus.

The virus from the fetus was neutralized by the

maternal convalescent serum and not by her acute serum. And the virus from the mother compared to the virus from the fetal were both neutralized to the same degree by a convalescent serum from a third laboratory proven case of rubella. We feel this is interesting because the lapse of time between the onset of the maternal difficulties and the isolation of rubella virus from the fetus was 57 days.

(Applause)

DR. MEYER: Thank you, Doctor.

Are there any questions?

DR. KEMPE: Can you give us the dosage of gamma globulin?

DR. HEGGIE: It was 20 ML. That was given after the onset of rash.

DR. MEYER: Any other questions?

(No response)

DR. FELDMAN: I think it would be terribly important if somebody, perhaps some of you do have already cases where the rash or the clinical difficulties occurred just prior to conception. Again, if I go back to the old model we worked with, the toxoplasma, when this happens there is no infection of the fetus as one might expect. But it would be nice to be able to corroborate it with another.

You are almost at that point and it is a little difficult to understand actually when you think in terms

ISOLATION OF RUBELLA VIRUS FROM THE FETUS FOLLOWING MATERNAL RUBELLA

SUBJECT	WEEKS PREGNANT AT TIME OF RASH	WEEKS BETWEEN RASH AND ABORTION	RUBELLA VIRUS ISOLATED	GAMMA GLOBULIN
M.V.	7	1	YES	YES *
J.W.	8	1	YES	NO
M.P.	9	1	YES	NO
C.N.	6	3	NO	NO
L.B.	2	4	YES	NO
C.S.	6	4	YES	NO
B.Z.	2	6	YES	NO
C.V.	3	6	YES	NO

* 20cc GAMMA GLOBULIN ON DAY OF EXPOSURE

+ CLINICAL DIAGNOSIS OF RUBELLA CONFIRMED BY ISOLATION OF VIRUS FROM PHARYNX

of the fetus or the embryo just how the virus gets to the embryo itself.

If somebody can find one or you can get one where the infection occurs just prior to a case like this, where records exist for it, it would be very helpful. One would expect the embryo would be made infection free. It would be nice to corroborate it.

DR. HEGGIE: I have no such case.

DR. FELDMAN: I think it would be one to keep an eye open for.

VOICE: There is one in progress right now. Dr. Balsamo is studying one.

DR. MEYER: Dr. Balsamo.

REMARKS OF DR. BALSAMO

DR. BALSAMO: For the past year we have had the opportunity to study 12 specimens obtained by therapeutic abortion for first trimester rubella. All specimens were obtained by dilation and curettage. Thus far we have completed viruses isolation studies in eight of these specimens. All virus isolation studies were carried out by the interference technique utilizing the green monkey kidney. The results of virus isolation studies are summarized on the following slide.

(Slide)

As you can see the weeks pregnant at the time of the rash varied from 2 to 9. The weeks between the development of

rash and therapeutic abortion varied from 1 to 6. We were able to isolate the rubella virus by the interference technique in 7 out of the 8 patients. In patient CN, from whom we were not able to isolate the virus, she was seen at the time of rubella rash. She had the characteristic rash and lymphadenopathy seen in rubella and as the rash faded she developed what is described as rubella arthritis. This lasted for a period of 4 or 5 days and then cleared spontaneously.

So it is of interest that this patient in whom this diagnosis of rubella was definitely established we could not isolate virus from the time of conception.

I should mention also that most of the specimens obtained by us were a small fragment of tissue and we could not distinguish in most cases from fetal and placental tissue. The third patient on the list, MP, it was possible to clearly differentiate between placental and fetal tissue. Then the virus is isolated from the fetus and the placenta.

The next and last point of interest on this slide is that only one of the 8 patients received gamma globulin.

This patient was of particular interest because she had a very definitive date of exposure when her sister returned from college with a rash. She saw her obstetrician on that day and received 20 cc's of gamma globulin. Twenty days later she developed tyical rubella rash and she had a therapeutic abortion performed one week later.

It was possible as you see on the chart to isolate an interfering agent from the product of conception in this patient.

I should mention that the first two patients on the chart had their rubella and the therapeutic abortion performed in the spring of 1963.

The remaining six contracted their difficulties during the present epidemic.

Thank you.

(Applause)

DR. MEYER: There seem to be several questions.

DR. NOVAK (Syracuse): I wonder if you have been able to determine the level of antibody gamma globulin in that first patient?

DR. BALSAMO: We have not. This was done in the hospital outside of New York City proper and it was gamma globulin supplied by the Department of Health. We have other charts. I have data on the Department of Health gamma globulin which I will present later this morning.

DR. MEYER: Are there any other questions?
(No response)
Dr. Schiff?

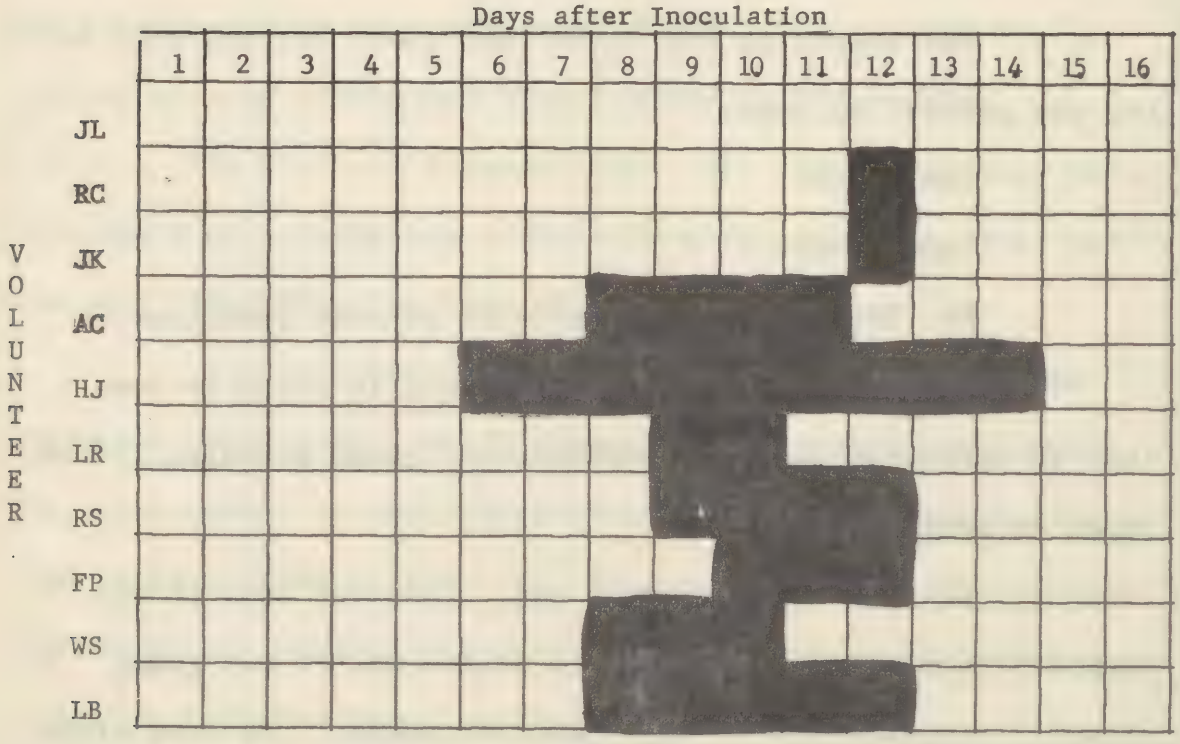
REMARKS OF DR. SCHIFF

DR. SCHIFF: Could I have the slide, please?

(Slide)

These are some results from human volunteer studies

RECOVERY OF RUBELLA VIRUS FROM BLOOD



which we have done over a year ago.

I show this to characterize the period of viremia in the patient. The patients have received an internasal inoculation of tissue culture passage virus. This is 100TCID 50, what we consider a high dose as compared to nature.

The rash occurred in these patients on the 12 date but we were able to recover virus from the serums as early as the 6 day. At the time of the rash, or when the rash had gone, the viremia had disappeared. At the same time anybody at low levels was detected, the antibody rose significantly over the next three weeks.

Slide off.

For this reason we were very interested to learn of the studies of Dr. Selzer where she had recovered virus from the fetus at a time relatively long after it seems to disappear from the maternal blood.

Soon after we were able to obtain the fetus -- that was last January -- the history of this fetus is as follows: from a patient, a 21 year old white woman, who had her LMP on the 30 of November, was exposed on the 5 of January, received gamma globulin six days later and developed rubella on the 18 of January or 13 days after exposure, seven days after receiving gamma globulin. Yet she received 12 cc's of gamma globulin. Two and a half weeks after the development of the clinical difficulties she underwent a D and C. We were able to be

present at the D and C, were presented with the scrapings. From this we were able to identify only a very small piece of fetal tissue, of fetal heart. This was washed carefully in Hank's, balanced salt, washed five times, frozen in Hank's with gelatine at the site of the procedure. Later it was made into a ten percent suspension, diluted in half log dilution and inoculated in the monkey kidney tissue.

Through the interference technique we were able to recover ten to the two logs of virus from this particular specimen. At the same time a piece of placenta or decidua, or unidentifiable description -- which we have received that cannot be identified as being fetal tissue -- was also inoculated and we recovered one and a half logs of virus. This is all the first, from the raw specimen.

A sera collected at that time of the D and C had an antibody of 1:16.

A throat swab at the time of the D and C was tested for the virus and also the sera was tested for the virus. Unfortunately we had no pre-bleed on this particular patient. We were quite impressed from the results of this particular adventure on the great chance of mixing maternal fetal tissue. So we then have placed most emphasis on getting the larger in toto fetuses. In recent weeks with the fine cooperation of the 16 hospitals and universities which are members of the perinatal research project and under the interested

obstetricians we have been able to collect 14 additional fetuses. Many of these, or the majority of these have been done by vaginal and cervical packing, hypertonic salt instillation of the uterus and "C" section.

There are several which have also been done by "D and C".

In most of these cases one of the personnel from our laboratory, one of the four divisions of our laboratory, were present at the procedure, were able to handle the tissues in a manner which would be most optimistic to recover virus. At the time of the procedure attempts have been made to collect maternal cord blood, blood from the fetal heart, amniotic fluid, placenta.

In most patients in the perinatal study, a pre-blood has also been collected. The results from these fetuses are not finished. Several are on test but none have been completely worked up. I can only report one additional finding, something which I think would add to the information by Dr. Alford.

This was a woman who had rubella in the second week of pregnancy and then seven days later underwent a C section on which materials were collected. At the time of procedure the fetus was delivered in toto. The fetal membranes were intact.

And so different materials were separated quite

carefully. From the amniotic fluid of this particular fetus we were able to recover a virus. None of the other studies have been completed.

(Applause)

DR. MEYER: Thank you.

Are there any questions on Dr. Schiff's presentation?

DR. PARKMAN: In that amniotic fluid were you able to titer how much virus was present in that?

DR. SCHIFF: This is being done. We have many analyses of this and we got only recovery first on this and we are now back on it.

DR. MEYER: That brings us down to Dr. Parkman.

REMARKS OF DR. PARKMAN

DR. PARKMAN: I would just like to mention briefly our experience with two human embryos in therapeutic abortion.

The first of these abortions was performed four and a half weeks after the onset of rubella during the first months of pregnancy. We confirmed the diagnosis of the rubella in the mother by ser o logic studies. We attempted to isolate virus from ten percent suspension from fragments of placenta, decidua and fetal tissue.

We were able to identify the fetal fragments as coming from a fetus of about 30 to 40 days by the facial characteristics of the fetus. We did not recover any viruses

RUBELLA IN PREGNANCY

Pt. M. K.
Onset of rash: 1/12/64
acute serum 1:2 (1/14)
conv. serum 1:8 (2/8)
Therepeutic abortion on 3/9/64
Rubella virus recovered from fetal fragments.

FETAL RUBELLA INFECTION

Tissue 10% susp.	Virus Titer $\log_{10}$/gm of tissue
Face & eye	2.8
Mandible	2.5
Ribs	2.5
Foot & hand	2.1
Intestine	3.5
Liver	1.5
Placenta	0.6

from this first attempt in the greater monkey kidney or LLCMK 2 cultures.

May I have the first slide?

(Slide)

The second patient had the onset of rash in January of this year during the first month of her pregnancy. Sera drawn on the 2nd and 24th days of disease shows a significant antibody increase. On the 57th day after infection, or at about two and a half to three months of pregnancy, a therapeutic abortion was performed.

May I have the next slide?

(Slide)

This abortion was performed by a dilation and curettage. All specimens were collected in one container. Therefore all specimen were floating in the same fluid.

We were able to divide the fetal specimens into individual specimens. Each specimen was washed several times with cold balanced salt solution and made up as a ten percent suspension in balanced salt containing one percent bovine plasma albumin.

Each of these specimens was then inoculated into primary African greenmonkey kidney cultures. Virus was recovered from each of the fetal and placental specimens. No virus was recovered from the fluids which were collected at the outset of the procedure. This was mixed maternal blood

and amniotic fluid.

As you can see, the titers of virus per gram of fetal tissue ranged from 1.5 for delivered specimens to 3.5 for the small fragments of intestines and attached mesentery.

The virus titer from the placental specimens collected in the same container were significantly lower than those of the fetus itself.

And as I have said the initial fluid was negative for virus isolation. These by the evidence may suggest that the uniformity of virus titers is not simply the result of contamination of one specimen by another. If we can assume that this is true it appears that the infection of fetal tissues appears to be generalized and does not appear to be confined to those involved in the manifest congenital anomalies.

(Applause)

DR. MEYER: Thank you Dr. Parkman.

Are there any questions of Dr. Parkman?

DR. SEVER: Dr. Parkman, on the placenta, that has a lower titer than any fetal tissue?

DR. PARKMAN: Yes.

DR. SEVER: Then the mixture of embryonic fluid in maternal blood --

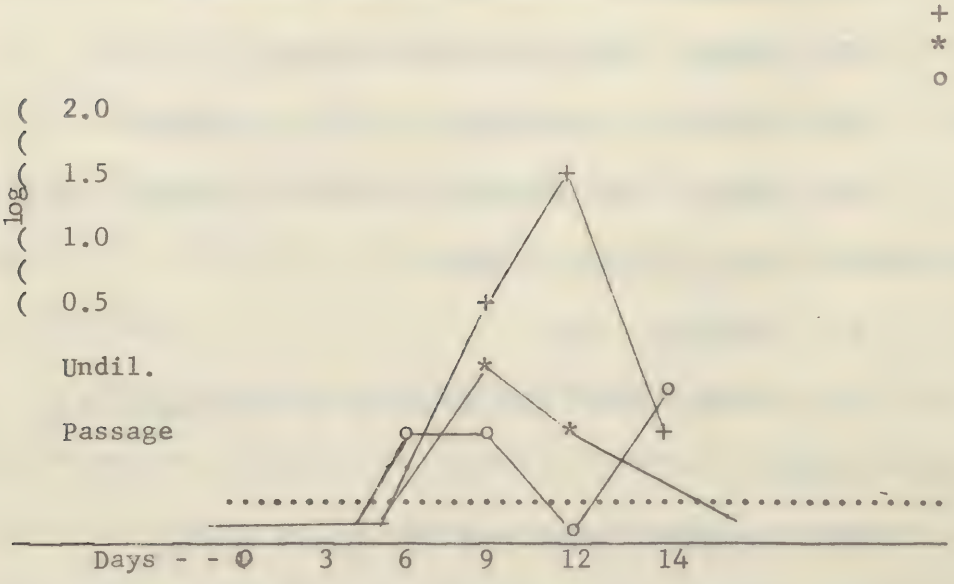
DR. PARKMAN: That did not yield virus.

DR. SEVER: I wondered if this might possibly be a function of the presence of high neutralizing antibody titer

Table 1. Experimental Rubella Infection in Ferrets
0.5 ml 50 $TCID_{50}$ RV strain, Passage 7-Intranasal

Day	Animal No.	Virus Recovery: Anterior Turbinate	Boney Turbinate	Lung	Serum
3	1	0	0	0	0
	2	0	0	0	0
6	3	0	0	+ (p)	0
	4	0	0	+ (p)	0
9	5	0	0	0	0
	6	+ (0.5)	+ (0)	+ (p)	0
12	7	0	0	0	0
	8	+ (1.5)	+ (p)	0	0
14	9	+ (p)	0	0	0
	10	+ (p)	0	+ (p)	0
	Totals	4/10	2/10	4/10	0/10

Figure 1: Rubella Infection in Ferrets



Study 3: Experimental Rubella Infection - Weanling Ferrets Sacrificed 12th Day

No.	Inoculum	Amount	Inoc. Route	Virus Recovery
1	6A	1 ml 100 $TCID_{50}$	IM	0
2	"	"	"	+ Turb.,Spleen
3	"	"	SC	+ Turb.,Spleen
4	"	0.3 ml 100 $TCID_{50}$	IN	+ Turb.,Lung,Spleen
5	"	"	"	+ Turb., Lung
6	Ferret Turb.	1 ml 100 $TCID_{50}$	IM	+ Lung, Turb.
7	"	"	"	0

Study 4: Experimental Infection in Pregnant Ferrets

Inoculum: 0.5 ml 100 $TCID_{50}$ RV Intramuscularly

Animal	Pregnancy Outcome Day 9	Day Sacrificed	RV Recovery
1	Spontaneous delivery-cannabalized	21	ND
2	"	"	ND
3	Spont. delivery-7 live born	10	+

Study 4-B: Recovery of Rubella Virus (RV) from Pregnant Ferret and offspring.

Animal	Day Sacrif.	Anterior Turb.	Boney Turb.	Spleen	Lung	Liver
Mother #3	10	+	+	+	0	0
Offspring #7	Age< 1 day	CNS/0	Rest of carcass/0			

Study 5: Experimental Rubella Infection in 2 week-Pregnant Ferrets. Sacrificed 10th Day

Animal	Inoculum Route	Inoculum Amount	RV Recoveries Uterine Contents	RV Recoveries Other
1	IN	0.3 ml 100 $TCID_{50}$	0	+
2	IN	0.3 "	0	+
3	IM	1.0 "	0	+
4	IM	1.0 "	0	0

Summary: Recovery of RV in Experimental Infection of Ferrets

	Route	No.	Recovery of RV	%
Weanling	IN	19	15	79
	IM	4	2	50
Pregnant	IN	2	2	100
	IM	3	2	67
Total		28	21	75%

Neutralizing Antibody Response of Ferrets
in Experimental Rubella

Inactivated Ferret Serum vs o.5 log RV

	No.	Route	Pre-Inoc.	Days after Inoc. 3	6	9	10	12	14	21
Weanling	10	IN	<1/4	<4	<4	<4		<4	<4	
	5	IN	"				<4			
	1	IN	"							16
	2	IM	"							16
										32
Pregnant	2	IM	"							8
										16

Study 2: Recovery of RV from Ferrets Inoculated with 0.5 ml of 100 $TCID_{50}$ Intranasally: Sac. Day 10.

Animal No.	Ant.Turb.	Boney Turb.	Spleen	Lung	Brain	Serum
1	+	+	0	+	0	Tox.
2	0	+	0	0	0	Tox.
3	+	+	+?	+	0	Tox.
4	C	C	C	+	0	Tox.
5	+	+	+	+	0	Tox.

in maternal circulation which might have been present?

DR. PARKMAN: Yes, this is certainly a possibility.

DR. SEVER: Would you venture a guess also as to the reason for the higher titer being in the intestine?

DR. PARKMAN: I really don't know why the intestine was higher. There was some attached mesentery so it is not really intestine, it is a mixture of tissues.

These were not pure specimens, certainly the extremities and the face specimens contained skin, muscles, and other tissues.

DR. MEYER: We seem to be in that peculiar position today as compared to yesterday of being way ahead of time. I am not sure which is worse. I believe Dr. Schiff if you can get your slides, we will go ahead with some of the work on experimental animals and take our coffee break in about thirty minutes or so.

REMARKS OF DR. SCHIFF

DR. SCHIFF: The availability of an animal model system for experimental rubella would be of particular value in the studies of the mechanisms of pathogenesis, of the disease, the development of vaccines for rubella, and the evaluation of newer anti-viral chemo-therapeutic agents.

In our laboratory, attempts to produce disease in mice, guinea pigs, hamsters, rats, rabbits, A.G. monkeys and baboons have failed. Similar attempts in rhesus and squirrel monkeys have yielded some early encouraging results, but to date these have not been confirmed.

The ferret was incorporated into our rubella studies following the report by Coates and Chanock describing the use of this animal with respiratory syncytial virus.

If I could have the first slide.

(Slide)

This is the ferret. The ferret is a member of the weasel family. It is omniverous, it eats fruit, berries, dog food and careless investigator's fingers. It has been used for centuries to hunt rats and mice, to rid buildings of rats and mice, also small field rodents and rabbits. In addition, they have been used in laboratory research in connection with canine distemper virus, influenza, rubeola, dental hygiene, reproductive cycles, and epilepsy.

Slide off.

For these studies six week old weanling and pregnant ferrets were obtained from the Gilman Marshal Company. The inoculum used was our laboratory strain of RV rubella virus. This virus was initially isolated from the ferrets of a six year old boy within 24 hours of the appearance of rash. It's been kept the last two years in tissue culture in our

laboratory. The animals were pre-bled, inoculated, and then sacrificed at various intervals up to 14 days.

At the time of sacrifice, the organs were removed, placed in a ten percent suspension, and tested for virus with the interference technique.

Several ferrets were allowed to live up to 21 days, at which time they were bled for antibody levels.

Again, the antibody titrations were done with the interference technique.

All specimens were passaged three times before being considered negative. All isolates were identified with specific hyperimmune rubella antisera.

Portions of the organs removed at the time of sacrifice were examined histologically.

To date, five studies have been performed. In none of these have the animals appeared to be clinically ill. Although the studies are not complete to date, no pathological lesions have been seen on the histological sections.

May I have the next slide, please?

(Slide)

Now, study 1, ten weanling ferrets were used. They were inoculated intranasally with our passage 7, seven times in monkey kidney virus. As you see, a total of half a milliliter of $10^{ID}50$ intranasally, .25 ML's in each nostril. Then at three-day intervals, except for the last interval two

animals were sacrificed. From these, on the third day we were unable to recover virus. Now the term anterior turbinate refers to the epithelial tissues overlying the turbinates; the bony turbinate was underneath, and also a little bit posterior to the epithelial tissue.

On the sixth day with one passage we were able to recover virus from the lungs of both of these animals.

On the ninth day we were able to recover a half log of virus from the anterior turbinate. An undiluted specimen yielded virus from the bony turbinate and again it took a passage in the lung.

On day 12 we got virus out of turbinate; at 14, out of turbinates and one lung.

Could I have the next slide, please?

(Slide)

Some attempt to show growth of virus. The dotted line is the threshold of ability to recover from a specimen. Passage means it took one passage for the virus to come out.

The anterior turbinate samples seem to yield the greatest amount of virus.

On day 12 we got one and a half logs. The bony turbinate seemed to yield second most and then from the lung it would move up in amount. It also took passage to get material out of the lung.

Next slide, please.

(Slide)

We then got an additional group of five ferrets in. We gave them slightly higher inoculum intranasally; sacrificed all on day 10.

Then we had recoveries from the turbinate; this time from the spleen from the lung; no virus was recovered from the brain.

We were unable to recover virus from the serum.

I might go back: In the first experiment we tried for serum and we were unable to do so. Unfortunately, this serum had been inactivated before we tried it. This time the serum was not inactivated. It proved quite toxic to our tissue culture despite steps taken to prevent the toxicity. This was a one to four solution. No virus from the brain.

We have also tested liver kidney without any recovery of virus.

Next slide, please.

(Slide)

Another group of weanling ferrets; these were all sacrificed on day 10. These received different inoculum. This inoculum is 6-A, the strain of virus which has been through nine passages in monkey kidneys and three passages in rabbit kidney. This titers six in rabbit kidney.

We gave some of these animals intramuscular injections. This also is a mistake, this also should be IM. From

these we recovered virus from the turbinates and supplies to the animals. We inoculated some animals intranasally with this inoculum and got isolations from turbinate, lungs and spleen. We also took a suspension from which we recovered virus from the turbinates of the ferrets in our first study, and inoculated intramuscularly and we were able to recover virus from the lung and the turbinate.

Now to date the titrations of these have not been completely finished, or not all specimens have been titered. There does not seem to be any increase in titer from these over -- they are all in the range of 10^{-1} and 10^{-2}.

Next slide, please.

(Slide)

The next two studies were gauged at trying to determine the effect of rubella virus on the ferret fetus. The gestational period of ferrets is 42 days. However, these are quite difficult to breed successfully, and are probably equally difficult to maintain in the early part of pregnancy. This is due to the temperament of the animal and also to the great increase in susceptibility to infection during pregnancy.

Now the first pregnant study involved three animals. While we had asked for animals early in pregnancy, as it turned out these were quite late in pregnancy. We inoculated these intramuscularly with this passage 7, seven times in monkey kidney strain. These delivered, much to our surprise,

much earlier than we had thought. They delivered sometime during the evening on the 9th day after inoculation. The mothers apparently were hungry and ate what they delivered. These were not sacrificed but were allowed to go on and were used for antibody studies.

The third mother -- if I could have the next slide --

(Slide)

was sacrificed the next morning or day 10. From her we were able to recover virus from turbinates in the spleen. Also from here seven offspring which were sacrificed on the following morning, on the morning of day 10, we tested the brains and the rest of the carcass minus the skin. These have been through three passages in tissue culture and have been negative.

The next slide, please.

(Slide)

The final study, which has not been completed yet, from the isolation point of view, involved four pregnant animals. They were again sacrificed on day 10. Two received intranasal inoculations of our passage 7 material in two IM inoculations. This time the pregnancy was interrupted on the tenth day. They appeared to be in their early pregnancy: the uterine contents were carefully removed and washed and ground up and tested for virus. This appeared through one tissue passage and has been negative.

From two of the mothers we have been able to recover

virus -- excuse me -- from three of the mothers virus from the turbinate and the lung or spleen.

Next slide, please.

(Slide)

This is a summary of the recovery of RV from our experimental rubella in ferrets to date.

This includes the two which we attempted to recover virus from on the third day. Overall we have 75 percent batting average.

Next slide, please.

(Slide)

This is a study of the antibody studies done to date. From the weanlings, those which were sacrificed, they were at the time of sacrifice also tested for antibody levels. They all were less than one to four on the pre-bled, but none of these which we sacrificed up to date -- 14 -- were we able to detect any antibody. The five which were allowed to go 21 days all showed at least a significant fourfold rise.

This is 21 days after inoculation. This is done in monkey kidney and rabbit kidney. This one goes up to 128.

Any questions?

DR. MEYER: Any questions regarding ferret infection?

DR. SEVER: I would like to add that in our studies with rabbits we have tested the products of conception after the intramuscular inoculation of rubella virus to the pregnant

rabbits approximately on the 6th day of pregnancy and in the one large series that we have completed tests we have not recovered the rubella virus from the material obtained at the terminus.

Of course these rabbits at that point, that extended the current study by injections directly in the uterus to the fetus.

DR. MEYER: Dr. Phillips?

DR. PHILLIPS: Have you doneany studies so far as contamination to find out whether this is transmissible from ferret to ferret?

DR. SCHIFF: No, we have not.

DR. MEYER: Since rabbits were mentioned again -- they were mentioned yesterday -- let me ask you: Do you have evidence that you can recover virus, ignoring the fetal rabbit, from the mother, can you get virus types from rabbit tissues, rabbit blood, or other types of material? In other words, what evidence is there, for how good the rabbit is as an experimental animal?

DR. SCHIFF: The studies to date using intranasal infection have not revealed as good results as ferrets, from the tentative findings which we have just received. Rabbits may be of some use; however, this needs to be explored in detail. We don't have the final information on that.

DR. MEYER: Dr. Buescher?

DR. BUESCHER: May I ask the strain of rabbit that is being used?

DR. SCHIFF: White rabbits.

VOICE: NIH rabbits.

(Laughter)

DR. MURRAY: If these are NIH rabbits this can be established.

DR. SEVER: No, they are not, it's Norwegian rabbits or something like that.

DR. MEYER: Let me ask Dr. Schiff a question, again turning to rabbits.

The reason we keep pushing this is I think the experimental host is something we are all very interested in, since this has been a real need in the rubella field, and there seems to be evidence that rabbits can be infected -- how useful it may prove to be remains to be seen. Has anyone had any evidence of communicability in rabbits? Has there been any work done on cage contacts or anything of this sort?

DR. SCHIFF: From our own studies we have had no evidence of communicability of rabbits. We have not seen the development of neutralizing antibodies in rabbits held in the same general area as infected rabbits. We have not done the very elaborate study that we have done with ferrets and other animals, and cages specifically set up to promote this type of investigation.

TABLE 1. GERMAN MEASLES VIRUS.

LYMPHADENOPATHY SCORES & SEROLOGIC RESPONSES OF CERCOPITHECUS MONKEYS INOCULATED WITH STRAIN M_{33} & OF THEIR CAGE CONTACTS

INOCULATION VOLUME & ROUTE	MONKEY STATUS INOC.	MONKEY STATUS CONT.	SEX	NO.	LYMPHADENOPATHY SCORE ING. L	ING. R	AXIL. L	AXIL. R	ON DAY	TITER ON DAY 30	TITER ON DAY 50
1.0 ml. IP											
0.5 ml. IN	X	X	M	561	+	+	+	+	10,11*	50	20
	X		F	567	+	+	+	+	10,11	13	10
		X	M	562	-	-	-	+	21,22	5	< 4
		X	F	568	-	-	-	-		< 4	"
1.0 ml. SC	X		M	563	-	-	-	-		20	5
	X		F	569	-	-	-	-		20	10
		X	M	564	-	-	+++	+++	15-25	< 4	< 4
		X	F	570	-	-	-	-		"	"
1.0 ml. IM	X		M	565	-	-	+	+	11	50	16
	X		F	571	-	-	-	-		20	10
		X	M	566	-	-	-	-		< 4	< 4
		X	F	572	-	-	-	-		"	"

* AXILLARY NODES SWOLLEN ON 11th DAY, INGUINAL NODES ON 10th OR 11th DAY.

TABLE 2. GERMAN MEASLES VIRUS.

INOCULATION TRACK, PARALYSIS & HISTOPATHOLOGY IN RHESUS MONKEYS INOCULATED INTRACEREBRALLY (IC) OR INTRASPINALLY (IS) WITH STRAIN M_{33}

INOCULATION ROUTE	VOLUME (ml.)	PROPORTION OF TOTAL NO. OF MONKEYS WITH SIGNIFICANT TRACK	SIGNIFICANT PARALYSIS	HISTOPATHOLOGY
IC	2 x 0.5	30/30	0/30	0/30
IS	0.2, UNDILUTED	4/6	1/4	0/4
	0.2, DILUTED 10^{-3}	6/6	0/6	0/6
	0.2, " 10^{-4}	6/6	3/6	0/6
IC	2 x 0.5, MEDIUM	5/5	0/5	0/5
UNINOCULATED CONTROLS		-	0/5	0/5

DR. MEYER: Any other questions on ferrets or rabbits?

(No response.)

Dr. Cabasso, could I ask you to go ahead and give your paper?

REMARKS OF DR. CABASSO.

DR. CABASSO: In our laboratory we worked with the RW strain kindly supplied by Drs. Weller and Neva and the M-33 for which we thank Drs. Buescher and Parkman. Because of the difficulty of distinguishing the rubella CPE, our work with amnion was limited. In some passages in human amnion, we saw the CPE described by Weller and Neva, but in other passages we could not distinguish the effects and we could perceive no change in the tubes even after 30 to 35 days of observation.

For all these passages the medium was that prescribed by Weller.

We had more experience with the M-33 isolate which was received, cercopithecus monkey culture, and the experiments that I will report were concerned with this isolate. In our early experiment we noted the results with rotated were better than the results with the stationary cultures and therefore rotated tubes were used in all experiments, including the neutralization tests.

Reports in the literature do not give a clear picture of inoculation of monkeys with virus. In 1914 Hess

reported that monkeys inoculated with rubella virus did not develop a rash but later someone differed -- in 41 rhesus monkeys inoculated with washings or blood from patients in the early stages -- 55 developed leukopenia.

Because of these variations a recent report by Sigurdardottir was of particular interest. Then these investigators used an inoculum with a titer of 10 to the 3.5 per one-tenth milliliter to inoculate two monkeys. A rash was observed on the 9th and 10th days. Instead of leukopenia these authors noted moderate rise in white blood cells with little or no change in normal temperature.

Our experiment was intended to supplement these observations. Since these did not yield useful information our experiment was only for rash and lymphadenopathy, and we also tested for antibody. We did not attempt to isolate the virus during the period. We used 12 Cercopithecus monkeys.

Slide, please.

(Slide.)

We used 12 monkeys, six males and six females. We inoculated one male and one female both intranasaly and intramuscularly. And with the passage of M-33 strain.

Each of these animals was caged separately and interesting in this stage was an inoculated monkey of the same sex in direct context with the non-inoculated monkey. We observed these for a period of fifty days and we saw no

rash whatsoever. On the examination of inguinal nodes and the axillary nodes in the two inoculated, we had a right axillary, slight lymphadenopathy in both the intraperitoneal inoculation and intranasal group.

In the contact groups we had, in addition to the monkey shown here, we had this monkey that showed a bilateral, slight paralysis. Others showed no clinical signs whatever.

On the 50th day the antibodies were still present in all inoculated monkeys but they had fallen to less than one to four in one monkey.

Well, the significance of this particular titer we do not know. However, the suggestion here exists that at least under the conditions of our experiment, using this strain of virus at the given passage level, we did not have any virus even after an intimate contact of these monkeys.

Lights, please.

Although rubella does not involve the central nervous system, it was intended to determine whether nerve cells would be invaded and destroyed when rubella was placed in or near them. To do this test we all performed the test exactly as a poliomyelitis vaccine. We used the same suspension, the same virus that had 10 to the 4 point 5 per ml.

The next slide.

We inoculated 30 monkeys intracerebrally and we

inoculated six monkeys intraspinally.

We observed these monkeys for temperature and any central nervous system disturbances and a variety of other parameters. There weren't any clinical symptoms to be observed during the day period. Except for probably this one out of the four monkeys that were inoculated with the undiluted -- these were then examined histopathologically by the same methods used by or for polio vaccine. As you can see, it was significant (indicating figures on slide).

Thank you.

DR. MEYER: Are there any questions of Dr. Cabasso?

DR. KRUGMAN: Did the last group of monkeys develop antibody?

DR. CABASSO: We did not look. We killed all at 18 days, we didn't have the serum.

DR. KRUGMAN: You killed them all?

DR. CABASSO: Yes.

DR. KRUGMAN: Were they free of antibody at the start?

DR. CABASSO: Again, we did not look at antibody at the start. But I was pleased to hear from Dr. Sever that the group of rhesus monkeys were 87 % antibody-free. They were held in quarantine and I suspect their level of antibody was perhaps of the same order.

DR. MEYER: On that first group you showed, in which

you showed the serology at 30 and 50 days?

DR. CABASSO: They were all negative.

DR. MEYER: That one contact that had the one to five titer, was negative?

DR. CABASSO: Negative.

DR. MURRAY: Did you make any attempt at virus isolation from the central nervous system?

DR. CABASSO: No, we did not. We have the frozen tissues, they are in storage. We may even attempt a virus isolation without having seen any lesions.

DR. MEYER: Dr. Kirschstein?

DR. KIRSCHSTEIN: I think one ought to be very careful in doing central nervous pathology, to realize that the polio was set up specifically to look for in the brains that were affected by polio viruses.

DR. CABASSO: We realize that and we looked at the cortex and other tissues as well. There was a total lack of any inflamatory or destructive lesions.

DR. MEYER: Is Dr. Warren still here?

(No response.)

I thought maybe he would want to make some comments on the monkey slide he showed yesterday.

I believe we can break now for coffee and be back in about 20 minutes.

(Thereupon, a short recess was taken.)

EXPERIMENTAL INFECTION OF RHESUS MONKEYS WITH RUBELLA

IMMUNITY STATUS	SEROLOGY		VIRUS RECOVERY FROM:		
	Pre	Post	Serum	Throat Swab	Rectal Swab
SUSCEPTIBLE	0/9*	9/9	6/9	9/9	4/9
IMMUNE	5/5	5/5	0/5	0/5	0/5

*Number Positive/Number Tested

RUBELLA RECOVERY FROM EXPERIMENTALLY INFECTED RHESUS MONKEYS

	DAYS POST-INOCULATION						
SOURCE	0	2	4	7	9	11	12-21
SERUM	0/9*	1/5	2/9	2/9	2/9	1/9	0/20
THROAT SWAB	0/9	0/5	3/9	5/9	6/8	5/9	0/20
RECTAL SWAB	0/7	0/4	0/6	1/7	1/3	1/7	2/18

*Number Specimens Positive/Number Specimens Tested

SENSITIVITY OF RHESUS MONKEYS TO RUBELLA INFECTION

TEST SYSTEM	LOG_{10} VIRUS TITER/1.0 ml
Monkeys	5.3
Tissue Culture:	
GMK	5.9
RK 13	5.4
BS-C-1	5.1

DR. MEYER: Gentlemen, if we could settle down now and get underway, please. We have one more presentation dealing with experimental animals. This one is by Doctor Phillips of our group.

I should mention, I think many of you know that Doctor Parkman has been working with Walter Reed and he is now in transit between DBS and Walter Reed, and I would like to stress that much of the data that has been presented by Doctor Parkman and will be presented by Doctor Phillips represents combined work from Walter Reed and DBS. The information on monkeys that Doctor Phillips will give is in that category. A great deal of this was accummulated while Doctor Parkman was working for Walter Reed.

STUDIES WITH MONKEYS

EXPERIMENTAL RUBELLA INFECTION IN RHESUS MONKEYS

DR. PHILLIPS: The need for an experimental animal susceptible to rubella infection is well recognized. Following on our work on rubeola in rhesus monkeys, we have been conducting experiments which lead us to believe that rhesus monkeys may also provide a satisfactory model for rubella infection in man.

May I have Slide 1, please.

A total of fourteen rhesus monkeys were inoculated intravenously with rubella in several experiments. The M33 strain, fourth Green Monkey kidney passage was used, and the

dose, measured in primary GMK tissue culture, varied from 2.4 to 4.4 log Interfering Dose 50.

Nine monkeys had no rubella neutralizing antibody before inoculation. All nine were susceptible to infection as demonstrated by sero-conversion during the third week after inoculation. The remaining five had pre-existing antibody and were classified as immune.

Virus recovery was attempted from all fourteen monkeys; sera, throat and rectal swabs were obtained at varying intervals after inoculation. These specimens were inoculated into GMK tissue culture and passaged once. Where interference was demonstrated, many of the isolates were typed with specific rubella antiserum. Rubella was recovered from all nine susceptible monkeys. No virus was recovered from any of the specimens from the five monkeys with pre-existing antibody.

Second slide please.

In this breakdown of the results, we see that the virus was most readily recovered during the second week after inoculation. This is where the great majority of the positive isolates were obtained. Only two isolates were made from 58 specimens taken after the twelfth post-inoculation day and both of these were from rectal swabs. The disappearance of the virus thus coincided with the appearance of neutralizing antibody. Rubella was most readily recovered from

throat swabs, sera, and rectal swabs, in that order.

It may be noted that these monkeys were observed for clinical signs of infection; however, at no time was overt disease, as manifested by fever, lymphadenopathy or rash noted.

There appears to be little natural immunity to rubella among rhesus monkeys. In testing 80 animals without known exposure, only 4 were found to have antibody.

Slide 3. Having demonstrated experimental rubella infection in rhesus monkeys, we wanted to determine their sensitivity to infection as compared with several tissue culture systems. Here 25 animals were inoculated intravenously with serial 10-fold dilutions of the M33 rubella strain, third BS-C-1 passage. Five monkeys were inoculated with each virus dilution. The inoculum was simultaneously titered in three tissue culture systems: primary GMK, RK-13 and BS-C-1. None of the monkeys had rubella neutralizing antibody prior to inoculation.

Seroconversion was used as an index of infection. These data show that the sensitivity of the monkeys to infection is of the same order as the sensitivity of the tissue culture systems.

In summary, rhesus monkeys appear to provide a promising model of rubella infection in man. They have a low incidence of natural immunity. Susceptible animals can be

experimentally infected as shown by the development of viremia, by the shedding of virus and by the appearance of neutralizing antibody. Pre-existing antibody appears to prevent reinfection.

Finally, monkeys appear to have approximately equal sensitivity to rubella infection as the present tissue culture systems.

Thank you.

(Applause.)

DR. MEYER: Are there any questions on Doctor Phillips' presentation? We have been interested in this question, the last slide Doctor Phillips presented, the sensitivity of the monkey as a possible experimental host as compared to the cell culture systems. This is of course basic information of value in considering vaccines and I am hoping the people from New York are going to be able to give us some sort of information concerning the titer of some of the rubella viruses in man, some of the tissue culture material. I don't know if you have this information or not, but it would be interesting to see how it does compare with the monkey in the cell titration system.

VOICE: You don't have any data I presume on material from patients? That is non-tissue culture adapted virus in the sensitivity of the monkey?

DR. PHILLIPS: No, we do not.

VOICE: I think it would be interesting to know this. The data Dr. Heggie obtained in Puerto Rico suggested that the monkey might be even more susceptible there than the tissue culture system.

DR. BUESCHER: Doctor Phillips, would you repeat again the passage level of the virus that was used in that titration?

DR. PHILLIPS: It was BS-C-1, third BS-C-1 passage.

VOICE: You made a point of the rather low incidence in monkeys naturally, and yet in the first series of 23 there were 9 with prior antibody. I was wondering --

DR. PHILLIPS: No, there were five with prior antibody.

VOICE: Which is still a significant number. Were they housed for quite a long time together, or were they collected from rather isolated conditions that might account for it ?

DR. PHILLIPS: No, these were animals we had in our animal rooms here, and had been here for sometime.

DR. MEYER: At least two of them, Paul says, I think at least two of them had been experimentally inoculated earlier. That is the reason they are positive.

VOICE: That is correct. Two of the five animals had been infected previously with rubella virus and at about three or four months later they were challenged with infectious

EXPERIMENTAL TRANSMISSION OF RUBELLA TO CHILDREN
· RELATIONSHIP OF SERUM ANTIBODY TO INFECTION

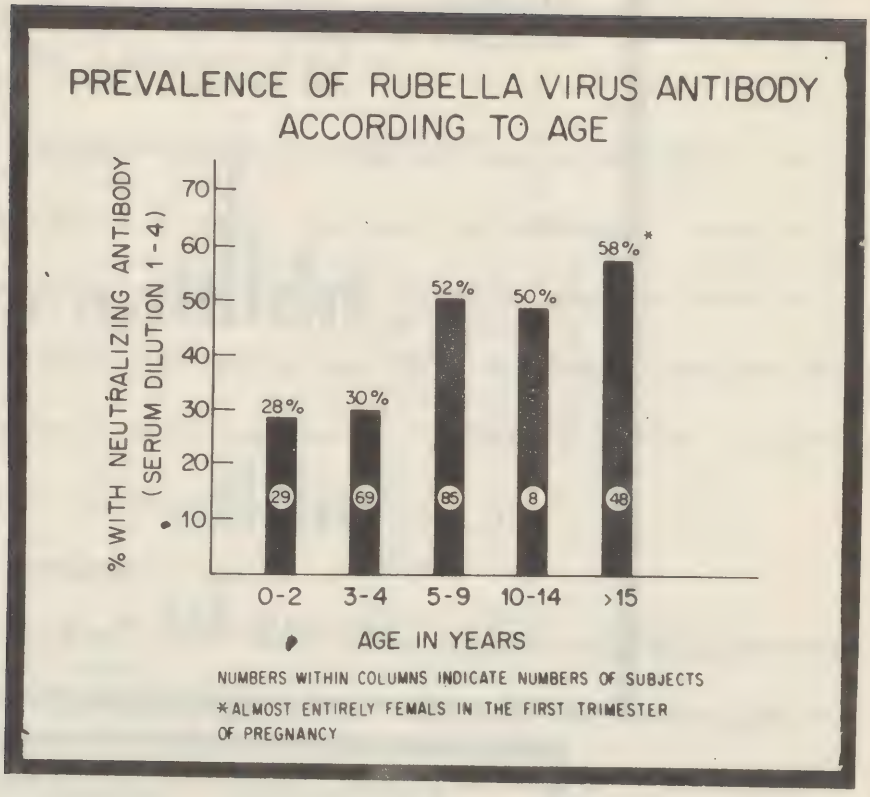
BASE-LINE SERUM ANTIBODY (1-4)	NO. OF SUBJECTS CHALLENGED	NO. WITH RUBELLA	NO. WITHOUT RUBELLA
ABSENT (SUSCEPTIBLE)	54	46	8
PRESENT IMMUNE)	37	0	37

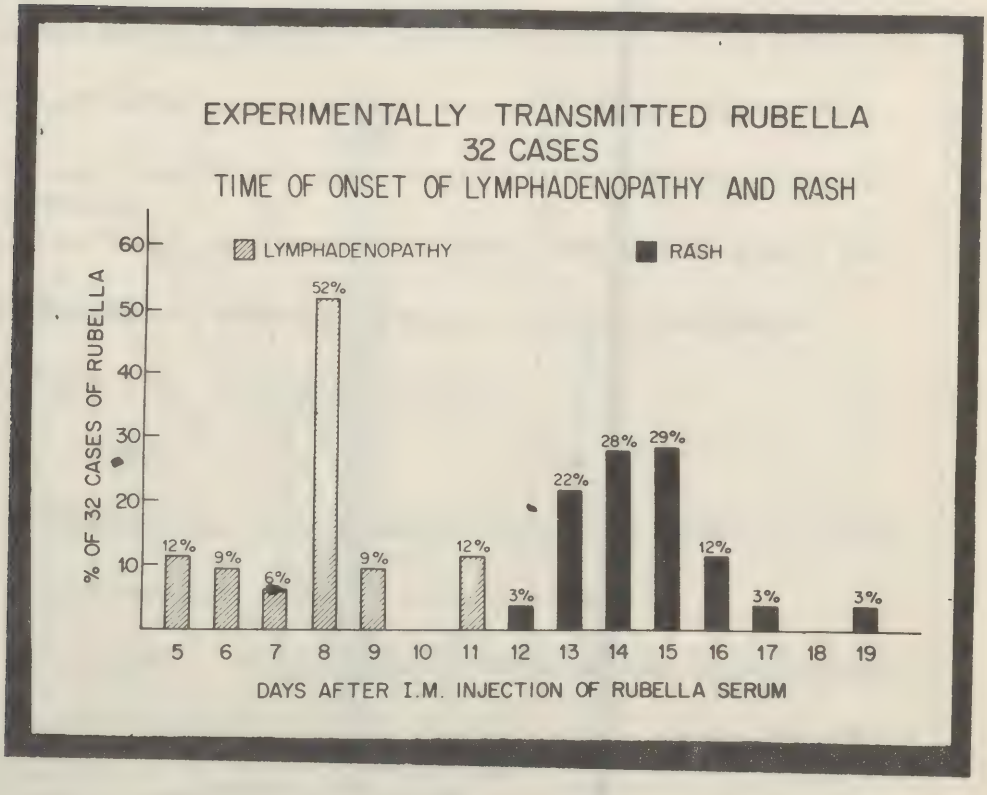
EXPERIMENTAL TRANSMISSION OF RUBELLA TO CHILDREN
BY VARIOUS TYPES OF EXPOSURE

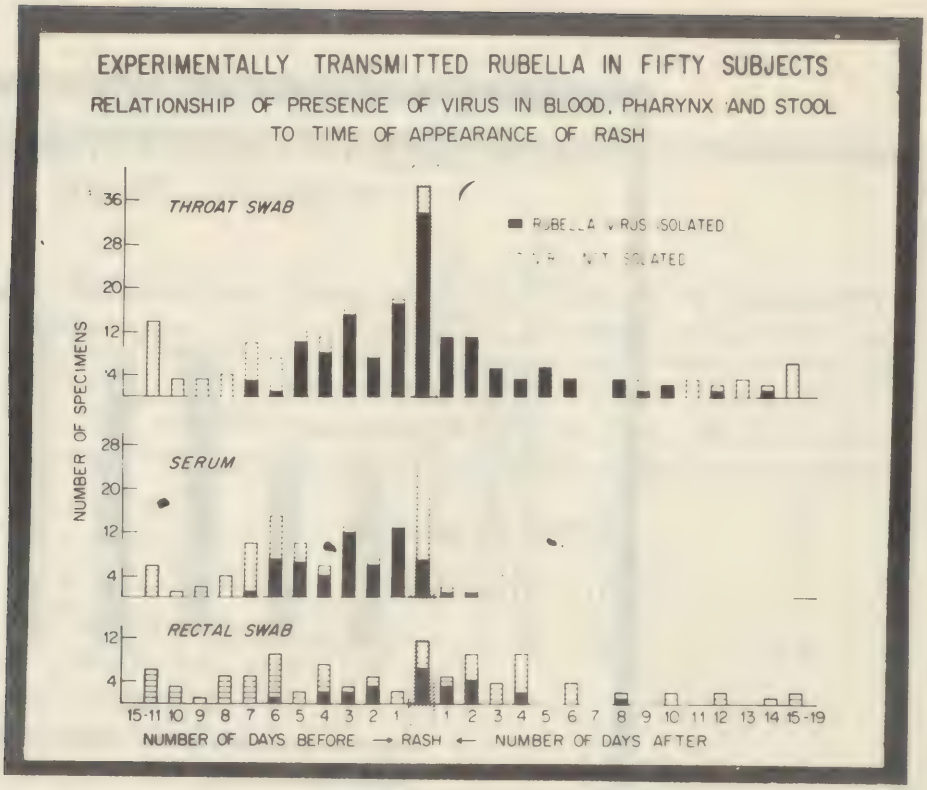
TYPE OF EXPOSURE	NO. OF SUSCEPTIBLE SUBJECTS	NO. WITH RUBELLA	
		CLINICAL [1]	SUBCLINICAL [2]
INTRANASAL INJECTION OF VIRUS	22	22	0
VIRUS SPRAYED	10	4	3
[illegible] (SPRAYED)	17	11	5
BRIEF [illegible]	5	1	0

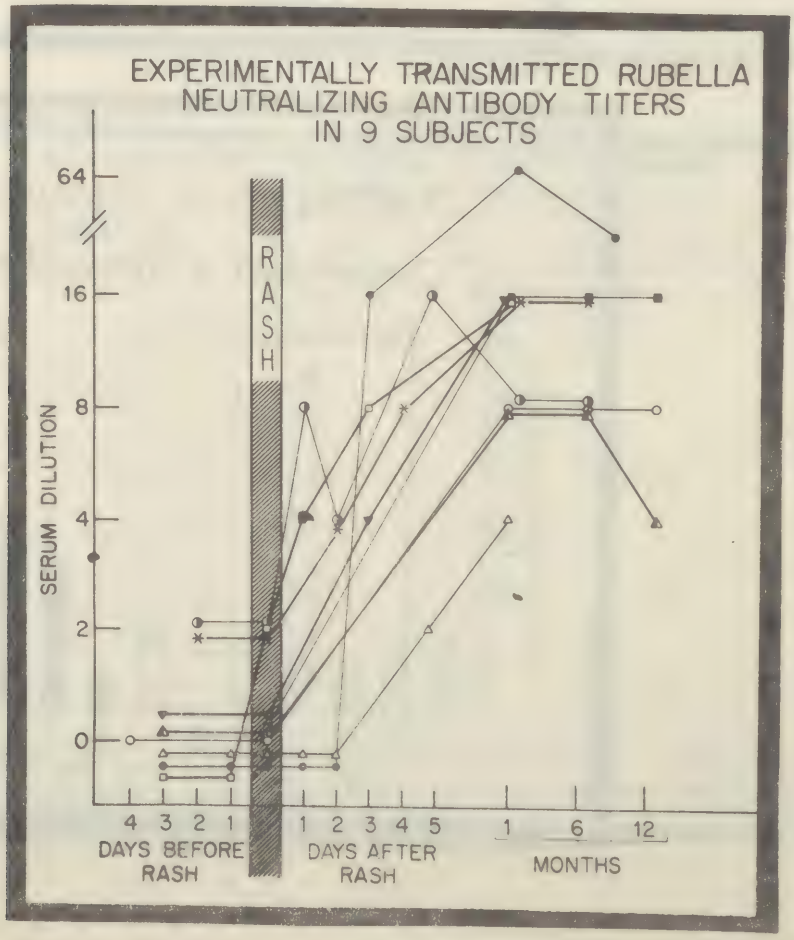
1 CLINICAL CASES: RASH AND ANTIBODY RISE WITH OR WITHOUT VIRUS ISOLATION

2 SUBCLINICAL CASES: NO RASH BUT ANTIBODY RISE WITH OR WITHOUT VIRUS ISOLATION


PREVALENCE OF RUBELLA VIRUS ANTIBODY
ACCORDING TO AGE
% WITH NEUTRALIZING ANTIBODY
(SERUM DILUTION 1-4)
70
60
50
40
30
20
10
28%
30%
52%
50%
58%*
29
69
85
8
48
0-2
3-4
5-9
10-14
>15
AGE IN YEARS
NUMBERS WITHIN COLUMNS INDICATE NUMBERS OF SUBJECTS
*ALMOST ENTIRELY FEMALS IN THE FIRST TRIMESTER
OF PREGNANCY


EXPERIMENTALLY TRANSMITTED RUBELLA
32 CASES
TIME OF ONSET OF LYMPHADENOPATHY AND RASH
LYMPHADENOPATHY
RASH
% OF 32 CASES OF RUBELLA
60
50
40
30
20
10
12%
9%
6%
52%
9%
12%
3%
22%
28%
29%
12%
3%
3%
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
DAYS AFTER I.M. INJECTION OF RUBELLA SERUM


EXPERIMENTALLY TRANSMITTED RUBELLA IN FIFTY SUBJECTS
RELATIONSHIP OF PRESENCE OF VIRUS IN BLOOD, PHARYNX AND STOOL
TO TIME OF APPEARANCE OF RASH
THROAT SWAB
SERUM
RECTAL SWAB
NUMBER OF SPECIMENS
36
28
20
12
4
15-11 10 9 8 7 6 5 4 3 2 1 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15-19
NUMBER OF DAYS BEFORE → RASH ← NUMBER OF DAYS AFTER


EXPERIMENTALLY TRANSMITTED RUBELLA
NEUTRALIZING ANTIBODY TITERS
IN 9 SUBJECTS
SERUM DILUTION
64
16
8
4
2
0
RASH
4 3 2 1
DAYS BEFORE RASH
1 2 3 4 5
DAYS AFTER RASH
1 6 12
MONTHS

virus. That represents two of the five.

DR. PHILLIPS: The other three had been housed here for a long time. Excuse me.

DR. MEYER: The question of communicability is of course of interest both in man and in monkeys. Especially in man as it relates to any tissue culture passage material. We deliberately have not given any data on this, because our data at the moment are inconclusive. I think, Doctor Cabasso, you were using 17 passage material?

DR. CABASSO: 25.

DR. MEYER: You were using relatively high passage material and in the experiment you reported on there didn't seem to be too much evidence of communicability. We hope to have a definitive answer to that shortly. It is possible earlier passage material, such as we are using, might be communicable and when the higher might not. Maybe the New York people can tell us something about man which would be even better than what we are finding out about monkeys.

Thank you, Doctor Phillips.

Dr. Green?

EXPERIMENTAL INFECTION OF MAN WITH RUBELLA VIRUS

Experimental infection of children.

DR. GREEN: This report concerns the experimental transmission of rubella to children which our group has been conducting during the past two years.

The titration originally described by Dr. Buescher and Weller and their associates on the African Green Monkey kidney system has been used exclusively for our virus isolations and in the antibody determinations.

First slide. Now this slide is a composite of a number of experiments in which attempts were made to transmit rubella to subjects, some of whom we didn't know were immune -- well, we didn't know their immunity status. And in some instances virus was inoculated and in others I suppose it was by contact. This will come out in the next slide. But I think you can see at once that individuals who had base line serum antibody of less than 1-4 were by and large susceptible. Forty-six out of 54, or about 85 percent contracted the disease in marked contrast to those who did possess such antibody; 37 in this group all uniformly resisted infection.

Next slide, please.

Now this slide illustrates the efficacy of several methods of exposure in transmitting the experimental disease. When virus in the form of infectious serum was injected intramuscularly, all of the 22 subjects developed classical rubella. When similar serum was sprayed into the nasal pharynx, it wasn't nearly so successful. Seven out of 10 developed rubella and three of these were sub-clinical cases. In other words, the diagnosis was a laboratory one, but they did not have a rash. Two types of contact exposure had been tried.

The idea here was to try to simulate the conditions that occur in nature, first to have prolonged or repeated contact that happens when the child or children of a pregnant mother contracts rubella and she is with them day in and day out for weeks, and here we took children infected with rubella and put them with groups of susceptible subjects and allowed them to mingle freely for a period of days. This was pretty effective, as you can see; 16 of the 17 susceptible subjects developed disease, but interestingly enough, five, or about one-third had sub-clinical disease.

Finally, the brief single type of contact that might occur when a pregnant woman meets a friend and chats with her briefly and then the next day her friend calls up and says "I have got a rash." Here this obviously is not very effective as a method of transmitting the disease. Only one of five such subjects developed rubella.

Next slide, please.

We have attempted to determine the prevalence of rubella virus antibody according to age. I should say at the outset that these are highly selected groups; virtually all of the children were institutionalized children, many of them had been there for sometime, although a fair number had come in from the outside.

In the greater than 15 year group practically all were young pregnant females who did not have a history of

rubella. But I think our figures here are somewhat higher than those that were shown yesterday. It seems to be some increase up to, oh, the 5 to 10 year group, then it seems to level off and there is not much difference after that.

Next slide, please.

This slide illustrates some of the clinical manifestations of the experimentally transmitted disease. The rash and lymphadenopathy were in no way different from that seen in the naturally occurring disease. These children by and large didn't have fever, they seldom did, and did not appear ill. After injecting the virus in the form of infectious serum, most of them got lymphadenopathy within about eight days and the rashes occurred about two weeks later, about a week later, or two weeks afterinoculation.

Now in the disease that was acquired by contact, this was pushed over about a week. In other words, the lymphadenopathy and rash actually occurred about a week later, which corresponds very nicely with the usual incubation period.

Next slide please.

Now as you have heard today and yesterday, it is apparently pretty easy to isolate virus from the pharynx, serum and stool of infected persons. Here we have compiled all of the data on 50-some subjects using the day of rash as a

focal point. One can see at once that virus is almost always present in the pharnyx on the day of rash and for a few days before and after.

In one instance, I guess in two or three instances, we found virus as early as seven days beforethe appearance of rash and in one instance as late as two weeks after the appearance of rash.

In contrast to this, virus is found most commonly in the serum for a few days preceding rash and on the day of rash there is a rather sharp drop, and after rash, after the day of the appearance of the rash, we have isolated virus in only two instances, one on the first day after rash and once on the second day after rash.

Now as you will see in the next slide, the disappearance of viremia corresponds very nicely to the appearance of antibody. Also virus has beenisolated from rectal swabs a number of days before and after rash. But as you can see this is pretty irregular.

Next slide please.

Here we are trying to demonstrate the pattern of development of antibody in the experimentally transmitted disease. These are nine subjects in whom we had serial bleedings with some interruptions. This antibody is almost always absent before rash and in a few instances it gets a low titer on the day of rash, but by and large it makes its

appearance two to five days after the appearance of rash. It then seems to reach a peak within a month and from what little data we have it would seem that the peak levels are maintained for at least a period of six to 12 months.

That is the last slide.

To go back to your question about degree of viremia, we have not done a great deal in this regard, but in some instances we have found, and Doctor Balsamo may want to elaborate on this, but we have found titers as high as 10 to minus 3.

DR. MEYER: Are there questions?

DR. ROBBINS: I think the question that Doctor Meyer raised was the relative susceptibility of the child as opposed to the tissue culture. Don't you have data on this?

VOICE: I think what the question is is how far the titration of infectious serum in children went as opposed to infectious serum in tissue culture and in children we had infection out to 10 to minus 3, whereas in tissue culture the virus was present only on second passage, it was not present in first passage.

DR. GREEN: Yes, there is quite a gap.

VOICE: Therefore the child was much more sensitive than the tissue culture system.

DR. GREEN: Yes.

DR. MEYER: This is working with infectious human serum as the source. You have no information on tissue culture material as the infecting source?

VOICE: No.

DR. WARREN: In the individual patient, when you compared antibody titer and carriage of virus in the throat, was there any correlation, because your slides suggest that you can have lots of antibody and still have lots of infectious virus persisting in the throat for sometime.

DR. GREEN: We have not actually analyzed our data carefully for that point, but I have the impression that that is true.

DR. HENDERSON: Henderson, Atlanta. I wonder if you could elaborate a bit as to what you mean by prolonged contact and whatyou mean by brief contact? What sort of prolonged contact was it?

DR. GREEN: We have actually two isolation wards at Willow Brook, and in the prolonged experiments, consisting of prolonged contact, we infected children by the intramuscular inoculation of known infectious serum and then we just turned them loose on a ward with a number of susceptible subjects and they stayed there during the entire experiment. In one experiment I think we didn't turn them loose on the ward, so to speak, until their rash had developed.

Now the brief contact, two subjects on the day of

appearance of rash, after they had been infected by intramuscular injection of serum, were allowed to kiss the susceptible subjects and that was it. They were just brought into the room and they kissed each other.

VOICE: Just once?

DR. RUEGSEGGER: Were any of your nurses ..... (?).....antibody before and after?

DR. GREEN: I couldn't hear you.

DR. RUEGSEGGER: Were any of your nurses or ward attendants tested for antibody before or after this?

DR. GREEN: No, we have not done this.

DR. RUEGSEGGER: Did you try to screen them for a history of it?

DR. GREEN: No, we haven't done it.

DR. KRUGMAN: In answer to this question, the same attendants and the same nurses and the same physicians have been present in this unit for the past year and a half and have been intimately exposed time and time again to natural rubella. I think this is an important point. And none have ever acquired any evidence of disease.

DR. SCHIFF: I can answer Doctor Warren's question partially with a slide here. Before I do that, about contacts, our own volunteer studies with adults, we had two patients who were antibody free, two volunteers antibody free in the same room with those who had the disease for a period of

eight days and these did not show any evidence of disease nor could virus be recovered or antibody conversion.

VOICE: They didn't kiss each other?

DR. SCHIFF: They might have.

DR. SEVER: I think it should be pointed out in those studies, tissue-grown virus in fifth or seventh passage was used.

DR. SCHIFF: Yes. This was fifth passage material. This is one of our volunteers from whom we recovered virus from the throat as early as the fourth day after inter-nasal administration, rash developed on the 12th day and virus was recovered up to the 21st day. On the 13th day we got an antibody level of 1 to 8 and then on the 16th day it was the same and about three weeks later it was up to 1 to 32. But this is the virus from the pharnyx, trying to quantitate it somewhat. As you can see at the time of the rash is where the virus is at its peak and then it drops off.

DR. MEYER: Doctor Feldman?

DR. FELDMAN: These are children whose personal hygiene may not be of the best?

DR. GREEN: Exactly.

DR. FELDMAN: And you got virus out of rectal swabs?

DR. GREEN: In a small percentage of cases.

DR. FELDMAN: Could this conceivably be a source of

infection for prolonged contact?

DR. GREEN: It might be. I suppose it could.

DR. FELDMAN: This would be quite different.

DR. MEYER: For those of you on the back row who couldn't hear Doctor Feldman, he is interested in the question of whether there is or there is not poor hygiene in the children and this might be a question of spread of virus from rectal material, if I got that correctly. Perhaps you gave this information, but I didn't catch it.

In your contact cases, the children who contacted either inapparent or apparent infection from your inoculated children, what was the ratio of clinically apparent infection in the contacts to inapparent infection in the contacts, getting back to this question we were talking about yesterday, if a child got contact infection, how frequently did he show clinical symptoms?

DR. GREEN: Well, I think it was in that slide, the second slide, I believe. With prolonged contact, there were 17 susceptible subjects, 16 developed rubella and five of the 16 had sub-clinical disease. It was almost one-third.

DR. MEYER: The vast majority had clinical disease. Is this clinical disease as defined by rash or by lympha-adenopathy?

DR. GREEN: Rash.

SUMMARY OF STUDIES OF THE PROPHYLACTIC USE OF GAMMA GLOBULIN

TYPE OF EXPOSURE	GAMMA GLOBULIN	TOTAL NO. SUSCEP. SUBJECTS	NO. WITH RUBELLA		NO. WITHOUT RUBELLA
			PRIMARY [1] CASES	SECONDARY [2] CASES	
VIRUS INOCULATED	0.12cc / lb	9	7 [3]	2 [0]	0
	NONE	13	10 [6]	3 [0]	0
PROLONGED (REPEATED) CONTACT	0.15cc TO 0.20cc / lb	11	8 [5]	2 [0]	1
	NONE	11	7 [2]	2 [0]	2
BRIEF (SINGLE) CONTACT	0.20cc / lb	4	0 [0]	2 [0]	2
	NONE	6	1 [0]	2 [1]	3

1 INCUBATION PERIOD: 12 TO 24 DAYS
2 INCUBATION PERIOD: 25 TO 53 DAYS
☐ NUMBER OF SUBCLINICAL CASES

VIREMIA IN CONTACT RUBELLA
N EVALUATION OF GAMMA GLOBULIN

NUMBER OF SUBJECTS[1]	GAMMA GLOBULIN	SUBJECTS WITH VIREMIA	NUMBER OF SERUM SPECIMENS[2]		
			TESTED	POSITIVE	
5	NONE	4	13	10	77 %
5	0.15cc/lb	4	19	7	37 %

[1] ALL SUBJECTS HAD CLINICAL DISEASE

[2] ALL BETWEEN DAY OF RASH AND FIVE DAYS BEFORE RASH

DR. MEYER: Doctor Henderson?

DR. HENDERSON: I wondered one other point I may have missed, what passage material was this you were using in the inoculating the children?

DR. GREEN: Well, it was serum obtained from a patient on the day of appearance of rash. We have done some tissue culture passage material. Doctor Balsamo may present some of that later.

DR. MEYER: Any other questions for Doctor Green? Thank you, Dr. Green.

Doctor Balsamo.

GAMMA GLOBULIN PROPHYLAXIS OF RUBELLA

DR. BALSAMO: The data I am about to present represents the result of six separate gamma globulin experiements carried out at the Willow Brook State School. In order to inform some of the people here about the Willow Brook State School, some of the procedures utilized there, I will briefly mention that there is a full-time pediatrician, primarily interested in infectious diseases, who examines each child in our study daily. Daily throat swabs are taken on each patient, as well as daily rectal swabs. Daily serum specimens are obtained at the expected critical period, critical periods that we have learned to expect from previous studies. Serum specimens are obtained at frequent intervals in other than expected critical periods.

We have performed actually three separate categories of gamma globulin experiments. The first category was a virus inoculation experiment in which infectious rubella serum was inoculated into a number of children. Half of these children received gamma globulin; one-half did not. In the next inoculation experiment, infectious serum was sprayed into the nasal pharnyx of a group of children. One half hour after the nasal pharnyx was sprayed, one-half of the group received gamma globulin and the other half served as controls.

The next category of experiments were the contact type experiments alluded to earlier by Doctor Green. In two of these experiments children were inoculated and exposed, in two separate experiments, children were inoculated and exposed to a number of subjects, test subjects, so to speak. Twenty-four hoursafter the development of rash in the inoculated patients, one-half of the test subjects received gamma globulin and one-half of the test subjects served as controls. This contact rubella or continuous contact rubella encompassed three separate experiments.

In the third experiment the children inoculated with, the so-called pilot cases were isolated until the day of rash. When they developed rash they were then exposed to the subjects who were going to serve as the test subjects. Twenty-four hours after exposure one-half of the children received gamma globulin, one-half did not. The last experiment

was the brief contact experiment mentioned by Doctor Green, and I don't think it is necessary for me to repeat his description any further.

Before I go into the details of our findings, I should mention that we have thus far tested 11 separate gamma globulin specimens and three gamma globulin pools. Using the interference technique for determining neutralizing antibody, four of these gamma globulin specimens have had a titer of one to 32, five have had a titer of one to 64, two have had a titer of 1 to 128.

I should mention that one specimen was obtained from Captain Miller. This gamma globulin was collected after a mild outbreak of rubella at the Great Lakes Naval Station in the fall of 1962. The titer on this material was 1 to 64.

We have also obtained specimens of gamma globulin from the National Institutes of Health and the titer on that material was also 1 to 64.

In the gamma globulin experiments I will mention now, gamma globulin with a titer of 1 to 64 was used in all experiments but one and in that first experiment gamma globulin with a titer of 1 to 32 was used.

May I have the first slide, please?

Just to refresh your memory about the three categories of gamma globulin experiments, in the first column here we have the virus inoculated group, which includes the inoculated with virus intermuscularly and the virus sprayed

into the nasal pharnyx. The middle column combines the three contact experiments, since there was essentially no difference in the results between the three separate experiments.

In the last horizontal column, the results from a brief contact are summarized.

In the inoculated subjects who receive .12 cc's of gamma globulin per pound, there were 9 susceptible subjects. Seven developed primary rubella as designated by a rash -- excuse me, as designated by an incubation period of 12 to 24 days. This was confirmed by virus isolation studies or antibody studies.

In the seven primary cases there were three sub-clinical cases. In the two secondary cases there were no sub-clinical cases and no child escaped.

I should mention that the small numbers i nthe boxes indicate the number of sub-clinical cases as is apparent from the slide.

In the control subjects in the virus inoculated group, there were 13 susceptible patients, 10 developed primary rubella and six of the sub-clinical variety. The three secondary cases were of the clinical nature.

In the so-called repeated or continuous contact experiment, the amount of gamma globulin administered varied from test to test, from .15 cc's in the first test to .20 cc's

per pound in the last two test groups. There were 11 susceptible subjects in the gamma globulin group and 11 in the non-gamma globulin group. Eight of the susceptible subjects received gamma globulin, developed primary rubella; five of the eight had sub-clinical disease. The two patients in this group who developed secondary rubella had clinical disease. One patient escaped as evidenced by no change in antibody titer, base line, or at time of discharge from the experiment isolation wards.

In the non-gamma globulin group, seven children developed primary rubella; two of these cases were sub-clinical. The two secondary cases were both of the clinical variety. Two children likewise escaped in this non-gamma globulin group.

The last column demonstrates the brief contact experiment mentioned by Doctor Green. There were four susceptible subjects who received0.20 cc's gamma globulin per pound; no child developed primary rubella, two children developed secondary rubella of the clinical variety, and two escaped.

In the six susceptible children who did not receive gamma globulin, there was one primary case and two secondary cases. One of the secondary cases was of the sub-clinical variety. Three children escaped.

I would like to draw your attention to the fact that

in our prolonged or repeated contact experiments, five of the eight primary cases of rubella were sub-clinical, whereas only two out of the seven non-gamma globulin primary cases were sub-clinical. This may be of some significance when related to the epidemilogical data presented yesterday.

In the last horizontal column, I think this type of experiment is of vital importance in order to evaluate the place of gamma globulin in the prevention of rubella and congenital malformations. It is obvious from this chart however that our attack rate was so low that we can make no conclusions about this experiment, and further work is obviously needed.

One question that arises frequently in gamma globulin experiments of this kind is the effect of gamma globulin on the development of viremia.

Next slide.

We have five patients who received gamma globulin and had extensive studies for the development of viremia. Five patients did not receive gamma globulin and were studied in a similar manner. I should mention these ten patients had clinical disease, throat swab isolations of the rubella virus and an antibody rise. All serum specimens enumerated on this chart were taken from five days before the day of rash up to and including the day of rash. There are two points of importance on this chart. The first point is that

we were able to isolate virus from at least one serum specimen in four out of five patients in each group.

The next significant point is that in 10 out of 13 serum specimens tested, in the non-gamma globulin group, virus was isolated for a percentage of 77 percent of the specimens tested.

In the gamma globulin group, only 7 out of 19, or 37 percent were positive. The significance of this data is not clear. I think the demonstration of viremia after gamma globulin given early is of some importance.

To summarize our conclusions about the gamma globulin data, it is I believe the consensus of opinion in our group that the gamma globulin that we have used in our studies has failed to be of prophylatic value in preventing the development of rubella in inoculated or in continuous contact exposure. The brief contact exposure is obviously of importance, but we have no definitive information in that regard. And the viremia chart stands for itself I believe. That is all.

(Applause.)

DR. MEYER: Captain Miller?

CAPT. MILLER: I just wanted to make clear, because this has been misunderstood in the past, that the material from Great Lakes was not collected from, was not selected necessarily from rubella patients, it was a general over-all

collection of about four percent of the donors that had rubella. And then one other point. I think it is going to be very important to see how well gamma globulin, how effective gamma globulin is in the preventionof viremia in the young adult, because we did not look for viremia, of course, in our gamma globulin prophylaxis on recruits and it is very possible, although we were preventing clinical apparent rubella, we were not preventing viremia.

DR. SEVER: I think these findings are most important for the problem of prevention of rubella in pregnancy and prevention of fetal defects relating to rubella. I am sure we are all aware of the extensive studies reported by Lundstrom and more recently the studies in the British Medical Journal on 6,000 pregnant women, both of these indicating that gamma globulin was effective in reducing the incidence of malformations. The studies presented today though certainly warrant extensive consideration and further investigation, I am sure, as you are probably planning to do in the study of natural infection in pregnant women with the availability of the techniques that are now at hand, I would imagine that an obvious extension of your studies would be to compare antibody status prior, and subsequent to the administration of gamma globulin to pregnant women and to correlate this information with the outcome of their pregnancy.

DR. BALSAMO: An epidemiological survey is now underway.

DR. CABASSO: This points up at least in my mind the necessity for those interested in gamma globulin to compare titers they get in the gamma gloculin and a necessity for perhaps in the early days to have a reference that people can test against, because the 1 to 64 titer mentioned here differs from the others and we don't know whether it is the same or a different level. If it were a true level, as compared to the levels obtained by others, it may be the reason for the lack of difference between the two groups. So it is essential to have, to know whether your titer is comparable to the titers obtained by others.

DR. BALSAMO: I believe some investigators have titered the same gamma globulin. Does anyone care to comment on that?

DR. MEYER: Just one comment about the reference. There is an obvious need for a serologic reference, I mean just in the course of comparing notes between various investigators, and there is a DBS reference volume available which can be sent to people on request. We were planning on mentioning that this afternoon. We can talk further about it then.

(VOICE)--- What is the titer of that gamma globulin in DBS?

DR. MEYER: Here again it depends upon your test system. With the test system Doctor Parkman described, again depending on the virus dose, about 1 to 100. It runs higher and lower, depending on the test.

DR. KEMPE: Doctor Balsamo, do you know the titer of Doctor Lundstrom's rubella gamma globulin?

DR. BALSAMO: He uses different units than we do apparently. I have never spoken to him about it. I don't know anything about the comparative --

DR. SCHIFF: We have compared his lot with other lots and his is higher than anything we have. Again figures don't mean much here, but his was 4,096 whereas our lowest was 236. This was a sample of material which he had used successfully clinically.

DR. KEMPE: I raise this point because this would be an ideal time, which may not happen again in ten years, for us to collect an experimental and perhaps clinical lot for clinical reference of rubella immune gamma globulin. In one small college in Colorado we have 200 young adult females who would be potential donors of a convalescent pool and I think between the people here it might be quite possible to get the 500 or 1,000 units that might be required to make a lot that would pass DBS standards and be used for clinical research. And this is something we might decide today, because the opportunity won't present itself again.

DR. BALSAMO: We have started or planned to do this on a very small scale, only in patients from whom we have isolated rubella virus from the blood or pharnyx. But this is a very small scale.

DR. MEYER: I would like to make one comment on this question of immune gamma globulin, which I think Doctor Parkman made some passing allusion to previously and others have commented on. One problem on convalescent gamma globulin or convalescent serum to make a gamma globulin pool is by far and large your convalescent titers in rubella are not significantly higher than the titers of many individuals you see in the ordinary population. We ran into this ourselves in trying to get convalescent serum pools for use in laboratory tests as compared to let's say ordinary serum pools. Doctor Parkman has as high a titer and he hasn't had rubella in years as the average convalescent person we run across.

DR. KEMPE: But Doctor Lundstrom's data are so much better than anybody else's and there is this difference in the titer.

DR. BALSAMO: This is what we found. The titer really doesn't change. You can't tell after the first two weeks of infection between a recent and late infection. One of the reasons we are interested in collecting gamma globulin, collecting this material from the patients who had a very recent infection is that perhaps there is some factor

other than neutralizing antibody that may be important.

DR. KEMPE: This may be the point. Maybe we are not measuring the right substance.

DR. MIRICK: I think one question was asked a short while ago, which I don't believe has been answered, and that is how the titers obtained by Doctor Belsame compared with titers on the same specimens obtained elsewhere. And there was one which was checked I believe by you, Doctor Parkman, where it was the same, 1 to 64.

DR. KRUGMAN: I think in this discussion it is important to think not only of titers of gamma globulin, but to re-emphasize a point made by Doctor Green earlier, when he presented the slide which showed that the virus that is present in the pharnyx as long as seven days before onset of rash. And this epidemilogical fact plus the observations made and reported by Doctor Balsamo, of no protection at all following prolonged intimate contact, I think is very clear, at least to me, that given this type of intimate household type of contact, the gamma globulin, no matter what the titer is, is given too late. It is given in all probability after infection has been established.

As far as the brief contact is concerned, we need more data about that. But it would be very -- we have had a number of instances in clinical practice, under controlled situations, where mothers have been exposed to their own

children and gamma globulin in one situation was given in a dose of 40 milliliters by a pediatrician to his wife and 19 days later this woman had classical rubella. There have been a number of instances like that. And this is not because of a titer of antibody, I think it is because of establishment of infection prior to the administration of the gamma globulin. As far as Dr. Lundstrom's studies are concerned, to the best of my knowledge these are not controlled studies. Doctor Lundstrom has given gamma globulin to large groups of pregnant women, but he does not have a comparable control group. I think it is very difficult to determine whether he really observed a reduced incidence of congenital malformation; unless you have information to the contrary I don't believe his studies are controlled.

DR. MURRAY: Apart from the possible use of gamma globulin prophylatically, which of course presents problems, I think Dr. Kempe's suggestion has considerable merit, because if we are going to rely on preparing a pool of gamma globulin, which will have laboratory value, this may be the time to do it. The epidemic seems to be migrating westward. I don't know how far it has gotten yet. But by contacting the various blood collecting agencies, it may be possible to get a large number of pools, donations of blood from persons who have had rubella within the past few weeks. The average pool of gamma globulin prepared commercially represents an average of

approximately 2,000 donations and if you could include in this 2,000 only those who recently had rubella, you might have material which has a somewhat elevated titer, which could have some scientific value.

DR. ROBBINS: I think the point also made by Doctor Kempe should be emphasized again and that is that we are going entirely on the basis of data with one kind of test, namely a neutralization test, and it may well be that when we get better, a different and diverse techniques, we will find that recent convalescent gamma globulin has antibodies that are quite significant.

Also I would agree with what Dr. Krugman said about the studies from Sweden. Although they certainly look suggestive as you go over the data of Dr. Lundstrom, if you talk to him you will talk to an evangelist who is firmly convinced himself, but certainly the data are far from conclusive in my humble opinion. The data that Doctor Miller showed from Great Lakes, I think it is well worth remembering that this was before exposure in a significant number of your recruits, so that this is not comparable to giving gamma globulin to exposed women.

Is that correct, Doctor Miller?

DR. MILLER: I think that is right.

DR. ROBBINS: This is truly prophylaxis of disease rather than --

DR. MILLER: It was given about ten days after arrival and I am sure some of the exposures had occurred prior to that time but the bulk were not.

DR. BALSAMO: We had one patient, a medical student, in which the gamma globulin was given seven to ten days before exposure and it didn't prevent any of the manifestations of the disease.

DR. WELLER: I would just like to second Doctor Kempe's suggestion that the time has come now to prepare some sort of a reference standard, I don't care whether it is gamma globulin or high titer convalescent serum, I think in terms not only of standardization of future preparations but in terms of asking each laboratory that works in the field to somewhere put down their titer of antibody that they achieve against this reference standard, thereby giving some reference for us all to cross check.

Now I would doubt a little bit from the information at hand that we need to make a convalescent pool. I would agree titers seem to stay up very well. We have impressive results with the Sindbis interference neutralization test, using 25 $TCID_{50}$ of rubella virus, in which four lots of gamma globulin were prepared and given to us by a Laboratory in Massachusetts, lots 1 and 2 were prepared in 1962 from 1500 to 1800 liter plasma pools, lot 3 was prepared at 54 from an 850 liter plasma pool and lot 4, and

Dr. Feldman may know something about the source of this, was prepared from bleedings of 137 patients in Ithaca and Syracuse, New York who had rubella in 1952, 7 to 18 weeks prior to bleeding.

Now the titers on these four lots were really all of the order of 1:1024. . In replica determinations it is true that lot 3 and 4 occasionally went as high as 1:4096. But the range in all of them was on the order of 10 to 24.

I think it would be extremely helpful if we could at this session decide to set up a common pool of material that we could all then use as a standard for antibody determinations, because we are going to want hopefully in the future to be studying antibody responses and being able to compare each other's results.

DR. BALSAMO: If I may make one comment in reference to the reference pool, we have tested at NIH lot 174, which I believe is the measles reference pool, and I think Paul has tested it and I would not be surprised if the NIH group has tested the same lot. So maybe we have comparative data already.

DR. KRUGMAN: I think the lot of gamma globulin, Tom, which you refer to, as being collected from Ithaca or Syracuse, is a lot which was prepared some years ago as convalescent rubella gamma globulin by Doctor Korns and members of the New York State -- is that the one?

DR. WELLER: That is the one.

DR. KRUGMAN: This is convalescent rubella gamma globulin. We used this material at Willow Brook State School in 1952, in a trial where it was mixed with serum known to contain rubella virus, and it was completely neutralized. This was also used in Taiwan during an epidemic of rubella. But it was used there and the quantity used in this trial was less than that of a commercially available lot of gamma globulin and from what you suggest this indicates I believe that the titer is no different from ordinary gamma globulin.

DR. WELLER: Of course we don't know anything about its storage history over the past 12 years and the stability of the material. So one might have to take this with a little bit of question. But as far as we can tell, these titers are high and regular pools have good high titers, within the sensitivity of the test. What this means one doesn't know.

DR. SEVER: I think to assist in resolving this question of having this material available, we prepared rubella from convalescent gamma globulin provided by recruits at Chanute Air Force Base last year. The total of approximately 150 units were included in this preparation and the gamma globulin made from that we have distributed to a number of laboratories on request. We would be very happy to provide the bulk of this material to DBS for further

distribution as one form of convalescent gamma globulin if this would be of assistance and if you wish to in that way have it available for comparative studies by different laboratories.

DR. BUESCHER: To go back to Doctor Kempe's original suggestion of a dual purpose, if there is question that processed gamma globulin can indeed be effective in prevention, I would like to raise the question as to whether or not part of this shouldn't be retained as whole serum. The question basically is would it be justified to evaluate the gamma globulin in the whole serum, the whole serum particularly, in such a circumstance as the New York group has.

DR. MEYER: Can I say one more thing? Before going further on this subject, this question pertains a bit I believe to some of the things we wish to discuss this afternoon, which basically is the reference standards. Perhaps we could be thinking about it between now and then and enlarge on the discussion of what might be feasible in the way of gamma globulin standards and collections in the afternoon session.

I would like to ask one more question before we move on to the next topic. We have had no data presented regarding the use in man of tissue culture passed rubella with the exception of one slide Doctor Shiff showed. Does

Nasopharyngeal Spray Of First Tissue Culture Passage Of Rubella Virus In Patas M.K. To Children

Dilution of Virus	Number of Susceptible Subjects	Number With Rubella	Number Without Rubella
Undiluted	4	4 (17 22 22 -1)	0
Diluted 1-10	4	3 (33 53) [1]	1
None	3	1 (63)	2

( ) = Day of appearance of Rash

[ ] = Subclinical Disease

anybody have any data concerning use of tissue culture passed rubella virus in man?

DR. BALSAMO: Yes, I think I have a slide on that. This material was first passage material in the patas monkey kidney. It was sprayed into the nasal pharnyx of 11 susceptible children. The titer of this material, it caused interference in the green monkey kidney tissue culture at a dilution of 1 to 10. I am sorry, it was sprayed into eight children, sprayed into the nasal pharnyx undiluted in four susceptible children, diluted 1 to 10, and four susceptible children were kept as controls. In the four susceptibles that received undiluted material, four of them developed rubella, and an incubation period of 17, 22 and 29 days. In all cases it was the clinical variety. In the four who received tissue culture material, diluted one to 10, three developed rubella at an incubation period of 33 and 35 days. One was of the sub-clinical variety. It is apparent that these patients most probably escaped who developed contact disease, but working with tissue culture material, we can't be sure about that. There was one escape in this group.

In the control children who did not receive the tissue culture preparation by nasal spary, one developed typical clinical rubella on day 63 and two escaped. It should be mentioned that two of the escapes in the control

children represent crib patients and it is possible their contact was not as good as the contact in the other patients.

We have since this time administered 25th passage tissue culture material with a titer of 4 and a half to a number of children at Willow Brook. But unfortunately three days after the experiment was started an outbreak of wild rubella occurred and the results are not interpretable.

DR. MEYER: Any questions on this last slide?

DR. WARREN: This is -- before we leave the matter of experimental infection, has anybody any information on whether the immune adult, in intimate contact with a sick child, carries live virus? Can you isolate live virus in the nose and throat?

DR. BALSAMO: I can't comment about adults. I can only comment about children. Immune children, we have not been able to isolate the virus from immune children, after close intimate contact with infected children, after nasal spray or intramuscular injection.

DR. PLOTKIN: In doing a fairly large amount of diagnostic virology, which we have been forced to do recently, we have tested a number of pregnant women who have been in contact with their own children or in some cases with other children, who had frank clinical rubella and on no occasion have we isolated virus from such a woman when there has been a neutralizing antibody titer in the first serum specimen we

had, which has usually been early in exposure.

DR. BALSAMO: I take back what I said earlier. I forgot we also have been running a viral diagnostic laboratory for pregnant females in New York and we have exactly the same results.

DR. MEYER: And these are the same results Doctor Buescher reported yesterday. I have forgotten the number involved. But the recruits that got antibody to start with, didnot have a shift in antibody, they did not excrete virus.

DR. BUESCHER: That is correct. They didn't have virus intheir throats by the schedule that was used for sampling.

DR. BALSAMO: One other word about sub-clinical patients. Isolation from the throat swab in sub-clinical patients are prolonged, even though no manifestation of the disease is present. We have isolated virus in as long as 14 consecutive days, without clinical manifestation of the disease.

DR. SHELOKOV: Was there a rise in the antibody content of the serum of these pregnant females?

VOICE: We have not identified any rise in titer in someone with a titer in the first specimen. We have of course had rise from infected people.

DR. SHELOKOV: But not the ones with pre-existing antibody, no rise?

Neutralization of Rubella Virus (RV) by Paired Serums of Rubella and Control Patients

Patients	Neutralization	
	Acute	Convalescent
Fort Ord, Calif., rubella (recruits)		
1	- *	+ ▼
2	-	+
3	-	-
4	-	+
5	+	+
6	-	+
7	-	+
8	-	+
9	-	+
Virginia, rubella (pediatric)		
1	-	+
2	-	+
3	-	+
Fort Ord, Calif., normal controls (recruits)		
1	+	+
2	+	+
3	+	+
4	+	+
5	+	+
6	+	+
7	-	-
8	+	+
9	+	+
10	+	+
11	+	+
12	+	+
Fort Ord, Calif., postrubella (recruits)		
1		+
2		+
3		+
4		+
5		+
6		+
7		+
8		+
9		-
10		+

* Screen neutralization with 50 and 500 $TCID_{50}$ RV.

▼ Neutralization, no neutralization.

RUBELLA VIRUS (RV) NEUTRALIZING ANTIBODY

I. Response to Administration of Rubella Virus (RV) and Control Fluid to Human Volunteers With and Without Pre-existing Antibody

A. Rubella Virus*

Volunteer	Neutralizing Antibody		Clinical Rubella
	Acute	Convalescent	
1	-	+	+
2	-	+	+
3	-	+	+
4	-	+	+
5	+	+	-
6	+	+	-
7	+	+	-
8	+	+	-
9	+	+	-
10	+	+	-

* Intranasal, 100 $TCID_{50}$ of virus (Primary African green monkey-kidney-tissue cultures)

B. Control Tissue Culture Fluid*

Volunteer	Neutralizing Antibody		Clinical Rubella
	Acute	Convalescent	
1	-	-	-
2	-	-	-

* Primary African green monkey-kidney-tissue cultures

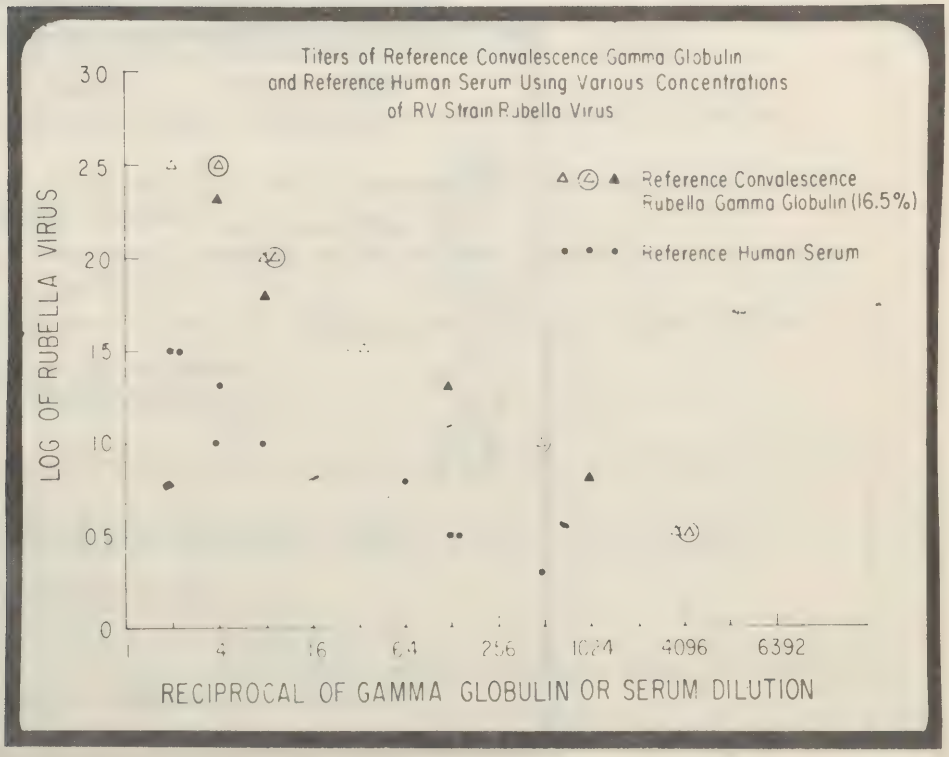


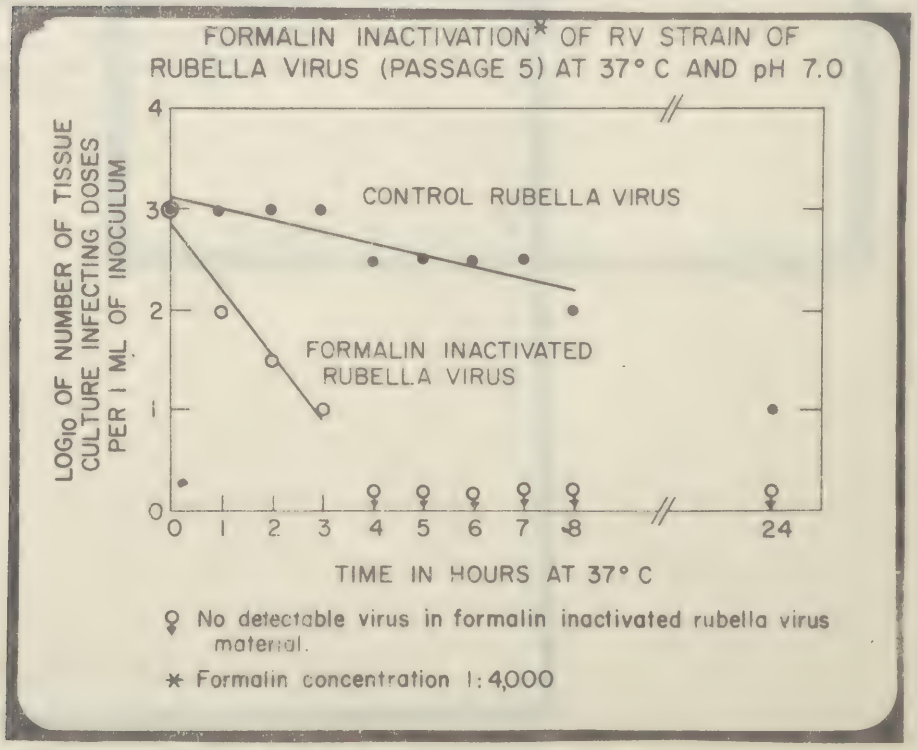
Table 2. Rubella neutralizing antibody in young adult males with natural and experimental infection.

No. of Cases	Neutralizing Antibody Titer*
Natural infection◆	
3	16
2	32
1	64
4	128
Experimental infection◆	
1	16
3	32
1	64
5	128
Natural infection (10 to 15 years earlier‡)	
3	16
4	32
2	64
1	128

* Determined with primary kidney tissue cultures from the African green monkeys, 0.5 to 1.0 log RV strain rubella virus; serial dilutions of serum; and 100 $TCID_{50}$ Coxsackie A-9 virus as indicator system.

◆ Specimens obtained 3 to 4 weeks after onset of rash.

‡ Date of rubella reported in case history.



VOICE: Not as yet.

VOICE: The same for the military population. When antibody is present and the individuals are exposed, there is no apparent change. During an 8-week interval.

DR. MEYER: Shall we move to the next section then and have discussion of Formalin Inactivation of Rubella Virus? Doctor Sever.

FORMALIN INACTIVATION OF RUBELLA VIRUS

DR. SEVER: The following information summarizes some of our findings with formalin - treated rubella virus vaccine. These studies represent a continuing effort, some of which was reported at the meeting of the Society last year and some of which is still in progress at the present time.

Briefly the techniques involved in this series of investigations have been reported previously in our original observations with volunteer administered rubella virus which was reported in the Journal of American Medical Association and the techniques with gamma globulin and serum titers have been described in detail in the publication of Dr. Shiff's, in Science.

To summarize just something of the information going prior to the main part of this study, I would like to have the first slide please.

This data was obtained several years ago in studies of serum specimens from recruits at Fort Ord , California, children with rubella normal controls at Fort Ord , and

recruits at Fort Ord who were sampled only in a convalescent state, in which the history of rubells three weeks earlier had been given.

These initial determinations then using the virus interference method, showed the conversion of serum specimens from negative to positive in almost all of these patients who had clinical rubella.

At this time of course we were just working with new techniques and in additional studies reported at the same time, the serum from a number of different outbreaks was tested under a code. These included outbreaks in island populations as well as in this country, and the reproducability of the test run at 1 to 4 for the screening of antibody titers like this was a little over 90 percent. The cross testing of course also demonstrated no serological difference between the various isolates of rubella virus.

Now subsequent to this, after extensive work in monkeys failed to demonstrate the clinical findings which we had hoped to be able to use for these studies, the first volunteer studies were initiated after review by the medical board here at NIH and the Director of NIH and these studies were done in conjunction with Dr. Shiff and Dr. Huebner, and the Federal Reformatory System.

Next slide. In these studies volunteers did not have the technical antibody, four of these men, and in a combination of two studies, for divergent purposes individuals

with pre-existing antibody were challenged with 100 TCID 50 of fifth passage primary African green monkey tissue cultures. This was given in a one cc inocculation internasally. All of the men negative for antibody developed clinical rubella. Clinical rubella did not occur in any of the individuals with pre-existing antibody. And the antibody conversion occurred in the volunteers who were susceptible. The virus could be re-isolated from the nasal pharnyx of course of these individuals, clinical rubella in this way occurs on the 11th to 12th day with the internasal administration of this quantity of virus. Virus isolations from the nasal pharnx can be made for a period of approximately a week prior to the appearance of clinical rubella and virus persists in the nasal pharnyx of these young adults for at least a week in many instances.

The period of viremia is for approximately a week prior to the occurrence of clinical symptoms and then disappears very shortly thereafter. This information was summarized on the slide Dr. Shiff showed earlier.

Viruria occurs for approximately the same duration as viremia, namely, for roughly a week prior to the occurence of clinical symptoms, and disappears shortly after the appearance of clinical symptoms.

Two volunteers included in these studies did not have neutralizing antibody in their first specimen. They were

given the control tissue culture material, which was tested in the same way as the initial material, and there was no conversion and no evidence of clinical rubella. This is a control of course simply on the presence of possible undetectable agents in the tissue fluids which might conceivable produce serological changes and clinical symptoms indistinguishable from rubella. This of course did not happen.

The titrations of antibody which I am going to refer to involved primarily determinations with the interfering method, which has been described previously, and secondly, we extended these and reworked all of the previous titrations and added on a number of other studies, using the RK-13 system.

Next slide please. In this data, this is a slide of the paper of Dr. Shiff's of course, the amount of virus used in these titrations is 0.5 log, and the reason for this selection is the reproducibility of the tests handled at that level and the fact that it is possible to make a good separation of titers between low and high titered gamma globulin and low and high titered human serums, so the test has value for separating it out; when one runs into a sensitive range you can block out and dampen this effect by adding more virus to the system.

Another advantage is that serum specimens screened at one to 4 have consistently been useful, using this amount

of virus, since individuals without antibody have been resistant to either natural or experimental disease, and individuals with antibody, I am sorry, individuals without antibody have been all susceptible and individuals with detectable antibody at the 1 to 4 level, using this amount of virus, with antibody, theyhave been resistant, without antibody they have been susceptible.

The titers obtained with this system, if we can have the next slide please, here again from the same paper with natural infection the titers are in the range of 16 to 128. With experimental infection, titers of 16 to 128. With natural infection, 10 to 15 years earlier, again the titers seem to be persisting quite unchanged, at least during this time interval.

These of course were identified as natural infection by history, since obviously the techniques have not been available to document the occurence of rubella that long ago.

Interestingly, the titers obtained with this method then, when run on RK 13, as reported previously, using 100 TCID 50, are quite comparable, and data between the two tests can be compared quite readily, the titers being within usually a two-fold variation of the titers determined in the African Green system.

Now the determination of inactivation of rubella virus with formalin is given in the next slide.

These tests were done with rubella material which initially had approximately three logs of rubella virus. The time in hours is given across the bottom. At 37 degrees, with formalin 1 to 4,000, there was no detectable virus at four hours; detectable virus was still present, one log, at three hours. But after that period it was not possible and of course the time has been extended here past 24 hours, there was no detectable virus. There is some thermal inactivation seen by the other line and this occurs with virus stored at 37 degrees centigrade. This has been found by a number of other investigators.

Our interest in this material, of course we were wondering if it would be possible to study this for its possible use as an antigen, formalin treated material for use for rubella. Our studies were first conducted in a series of experimental animals.

Next slide please. Now in these studies with rabbits, two separate preparations of the formalin inactivated material of the fifth passage in primary African Green monkey kideny tissue cultures is given. The material had been inactivated for a period of six days at 37 degrees centigrade. The initial titers obtained with one injection -- this is two weeks after the first injection and then the second injection was given at two weeks, just after the bleeding was obtained from these animals, and a final bleeding was obtained then three weeks after the second injection. Therefore the titers

are after one injection, taken two weeks after, and taken three weeks after two injections. In the rabbits, the untreated material gave titers in excess of 512. The loss or decrease in antigenicity occurred primarily then during the intitial six hours of treatment until the titers got down in the range of 16, 32 and 128, even 256, in these instances. It would appear then that after the initial six hours that there is also a slow decrease in antigenicity through the 8th day as indicated particularly by the specimens taken after one injection of this treated material, and yet this decrease is considerably less rapid than the initial decrease, which is first seen. Antigenicity in these two preparations persisted in the material through eight days of formalin treatment. Of course bisulfite was added at various periods, the material was stored and refrigerated during the period of treatment for subsequent studies.

Further studies were done with the material, particularly that at six days, simply because this was well after the last detectable virus was present. Studies were done in monkeys and a number of other species of animals. Titrations were done initially of course in the interference system and also thensubsequently in the RK-13 system. These titers presented here incidentally are specifically those from the RK-13 determinations; one could superimpose the other titers for the most part within two-fold when determined in the African

Green system. The animal titers were quite similar to those, the findings were quite similar to those found here in rabbits. In monkeys, antigenicity was demonstrated and the titers were in the range of generally 8 to 32. These titers are a little less than, in the monkey situation, than titers which we get frequently with untreated tissue cultures. With the availability then of antigenic material, which demonstrated its antigenicity, all studies were done with an intermuscular inoculation of one mililiter of preparation, with a demonstration of anitgenicity, and the lack of any detectable virus, and after the thorough safety tests paralleling those very closely that are stipulated for measles vaccines, it was decided to inititate studies in a small group of volunteers to determine the antigenic response and the protective value of this material.

Studies were specifically designed so that the antigenicity would be determined prior to challenge. And of course the study would have been terminated had the material not proved to be antigenic in the volunteers.

The first study is shown in the next slide. In this study there were three volunteers who did not have pre-existing antibody, who were given vaccine. There were three volunteers who did not have pre-existing antibody, who were given a control vaccine, control in quotes. It was identical to the vaccine material in its passage level and its material

but it had been carried as the control for the vaccine material, it was not inoculated. Two volunteers did not have pre-existing antibody and were given saline and two volunteers had pre-existing antibody, with titers of 64 and 128, and they were given control material. In each case the immunization procedure was two intermuscular inoculations, one mililiter each, given two weeks apart. Antibody titers then determined 14 days after the first injection and 35 days after the second injection demonstrated the development of neutralizing antibody in the individuals who were given the vaccine material. There was no development of antibody in the three individuals who were given the control material. There was not development of antibody in the two susceptible individuals who were given saline and when control material was given to two individuals with pre-existing antibody, the antibody titers persisted essentially unchanged. The demonstration of the development of neutralizing antibody then permitted the completion of the study.

I would also like to say these volunteers were checked essentially daily for the appearance of virus in the nasal pharnyx and other specimens after the administration of vaccine or control material. This was done to try to take advantage of what might be a more sensitive test for non-detectable virus in the vaccine materials. None of the volunteers demonstrated the appearance of live virus in the

nasal pharnyx and none of them developed clinical rubella following the administration of the vaccine material.

The challenge virus then was given and in this case here when the control group of two volunteers was included for a control challenge, and for this reason saline was given. The challenge virus was given internasally and this was 5th passage, RV strain rubella virus, 100 TCID 50, the same material used in the previous study I referred to, the three volunteers who received this challenge and had previously received vaccine did not show evidence of clinical rubella. The susceptible three volunteers who received control vaccine of course did not get an antibody response, after challenge, all developed clinical rubella, which persisted from the 12th through the 14th or 16th day. Two volunteers who got a control challenge and were susceptible did not show clinical rubella and two volunteers with pre-existing antibody, who were challenged with rubella virus, did not show clinical rubella. The virus was re-isolated from the throat specimens of all three volunteers-- I am sorry -- all three volunteers who were in the second group, the control group, the virus was isolated from two throats and there was no test on one, it was re-isolated from the blood of two, no test on one, and from the urine of the one that was tested. The virus could not be re-isolated from the throat, blood or urine specimens taken serially from the patients who had received vaccine, nor

from either of the control groups. The antibody titers, which were found 13 and 38 days subsequent to challenge, were essentially unchanged from the group which had received vaccine. Of course antibody developed in the individuals who had clinical rubella, antibody did not develop in the controls who were given a control challenge, and antibody persisted unchanged in those individuals who had pre-existing antibody and were challenged internasally.

Based on this preliminary study and after a review of the data we decided to extend these observations to a second study and this is shown in the next slide.

In this case only two materials were under study; that is vaccine was given to eight volunteers who were negative for antibody, at 1 to 4 and to five volunteer control material was given, to five who were also negative for antibody, 1 to 4. After the administration of the vaccine material, antibody titers appeared at 14 days after the first injection in all but one of the individuals and all of the individuals who received vaccine had antibody after the second injection. The administration of control vaccine material did not result in the appearance of antibody in any of the volunteers. All of the men then were challenged three weeks after the second injection with rubella virus. This was now 7th passage rubella virus, the other was 5th passage, 100 TCID 50, by the internasal route was given three weeks after the second injection.

None of them who had received vaccine developed clinical rubella, all of the ones who had received control material had developed clinical rubella. Virus could not be isolated from any of the specimens of the individuals whohad previously been given the vaccine material, it was isolated from all but one source from the five individuals who had received the control material; the neutralizing antibody titers subsequent to challenge were essentially unchanged in the group, which had received vaccine and of course antibody developed in the volunteers who had received control material and developed illness.

In these two studies 11 immunized volunteers were challenged with 100 TCID of rubella virus, three weeks after a two-stage immunization program. In this preliminary study all of the individuals were protected from clinical illness and virus could not be recovered from throat secretions, blood or urine taken after the challenge.

In the same studies, eight volunteers, without pre-existing antibody, who received control vaccine, developed rubella approximately 12 days after challenge. Additional studies now underway include the use of adjuvants and preliminary data from these studies in experimental animals indicates a four-fold or greater increase in antigenicity by the addition of alum to the formalin treated materials.

Studies on oncogenicity have also been underway

for a number of months. This involved the inoculation of newborn mice, hamsters, guinea pigs and rats, both with rubella virus tissue culture material and the inactivated material. After nine months of observation of all of the groups, there has been no evidence of tumors in any of the animals. It was interesting to find that the studies done in the second group of volunteers using 7th tissue culture passage rubella virus resulted in clinical illness which was more mild than that which occurred with 5th passage material. The rash was of shorter duation and less intense and in general the symptomology was definitely more mild.

The significant factors known at the present time include the sensitivity of the techniques, which are available and of course we are aware from the data of Dr. Krugman and his group that the tissue culture test systems are less sensitive than the similar administration of virus to children.

The data from the present studies using young adults failed to demonstrate any virus following the administration of the vaccine material in these adults. And although there is no clinical or laboratory evidence for the presence of live virus in the formalin treated vaccine material, the possibility remains that the sensitivity of all of these techniques which are available have failed to detect trace amounts of virus. We have no data available yet on the persistence of antibody, or protective effect after this

initial three -week period following the second immunization. Of course this is extremely important in considering a vaccine and further information on this must be obtained.

We have prepared a number of samples of materials and other laboratories have also done this, using the formalin inactivated procedures. I must say that not all of our preparations have had antigenicity, particularly when formalin inactivation has gone for eight or more days. And we are currently involved in extensive investigations on the various factors in the formalin treatment.

Further basic information is necessary of course to provide better information on the problem of rubella and rubella virus vaccines. We have used what are the best available tests for the detection of rubella virus, both in tissue cultureand in the swab material from the volunteers, and yet better assay methods are obviously necessary. We have employed both the interference method and the RK-13 method for the determination of antibody, and in our hands at the present time it would appear the RK-13 tissue culture system is most satisfactory and certainly considerably easier to use than the interference method. The further use of experimental animals for the testing of vaccinesnow seems to be at hand, since at least several animal systems are available and certainly these can be taken advantage of extensively in further studies certainly including monkeys and

ferrets , possibly rabbits. Studies on the teratogenicity of vaccine materials are most important and the studies which we have available now are being utilized and as of this time have not yet demonstrated the presence of oncogenicity potentials in the vaccine materials.

In general the future course of our investigation involves a expansion of our current studies on the antigenic materials and wherever possible now the utilization of commerciallly prepared materials in accordance with the usual recommendations and requirements of the Division of Biological Standards.

We have considered the use of other tissue cultures as a source of antigenic material and of course one could consider the use of primary and continuous rabbit kidney, dog kidney, baboon kidney and others. However, at the present time in our opinion there would seem to be no advantage in using the other systems over the African Green system, which we already know considerably more of the problems about.

Concerning attenuated vaccine, we have considered two approaches, attenuation in terms of teratogenic effects and communicability.

Since the first approach, namely the attenuation for teratogenic effects, can not be tested in humans, we have initiated studies on the second approach, dealing with attenuation of virus for transmissibility.

In a study just completed, we have completed among other factors the test of a 7th passage African Green rubella virus administered intermuscularly. This is the same material which gave a less severe illness when administered internasally tothe second group of volunteers which I presented. This was again evidence that the 7th passage rubella virus material gave the same degree of illness when administered intermuscularally as it did when administered internasally. The parenteral administration of this material produced clinical rubella in susceptible individuals, it was of the same intensity as the 7th passage material had been shown to produce when given internasally, and we feel therefore that the route had not markedly altered the problems for the tissue culture passed vaccine considerations.

Thank you.

DR. MEYER: We are now set for questions. Let me ask a quick one, Dr. Sever. In this 7th passage material you inoculated into man, both in the callenge study with your killed vaccine and also in the intermuscular inoculation, did you have any controls that could serve to show if the 7th passage material was communicable, man to man?

DR. SEVER: This was possible only in the study of 5th passage material. In that case as you remember there were two volunteers who had less than four antibody levels, and they were housed in the same room with the other men who

developed clinical rubella following the internasal administration of 100 TCID 50 of 5th passage material. These men with pre-existing antibody titers of less than four did not show any change in antibody, they also had antibody of less than 4 following the study, they did not develop clinical rubella. Now they were all together in one room roughly the size of this room, and they ate together, they played cards together, watched TV together, showered together, and under these circumstances we did not see evidence of transmission.

One of these volunteers could be included and was in the second study shown/which 7th passage was given internasally. This volunteer did develop clinical rubella when challenged with the internasal administration of the 7th passage material. The other man was lost to the study group.

DR. KRUGMAN: Doctor Sever, your demonstration of antibody, 14 days after inoculation of formalin treated material suggests very strongly that there may be some life in this material. I am impressed with the fact that in spite of the possibility of the material being live, as you indicate in your presentation, that these men had no symptoms and were completely asymptomatic.

My question is were the other controls in this group in intimate contact with these men? You indicated you don't have any data on persistence of antibody as yet. But perhaps you have attenuated this virus significantly, so

that it induces antibodies after 14 days and possibly doesn't spread. Were they in intimate contact and do you have evidence if there was life there, it didn't spread?

DR. SEVER: These men were in intimate contact, being in the room this size in individual beds equally spaced essentially throughout the room, and were there throughout the period which one would normally expect the virus to be present in the nasal pharnyx and subsequently were documented to have this occur in the susceptible individuals.

As I mentioned, they ate together and carried on all of the normal activities that a similarly confined group would carry on in this circumstance. They are in strict isolation from anyone else at the Federal Reformatory, they are in the hospital in an isolated room. There was no transmission, demonstrable, to these two susceptibles.

DR. KRUGMAN: I am talking about the inactivated group material.

DR. SEVER: There was no evidence of transmission of this. With the techniques which we have available we have been unable to demonstrate the presence of live virus. This is why we keep looking and hoping for more sensitive tests, for better systems. But as of the present time we can not detect live virus in the formalin treated material, treated in this way, using the tissue culture approach, using either interference or RK-13 or following administration of this to

Formalin Inactivation of Rubella Virus

Time	Titer
0	3.5 $\log_{10}$
1 hr	3.25 $\log_{10}$
3 hrs	1.25 $\log_{10}$
5 hrs	.75 $\log_{10}$
7-1/2 hrs	Not Detectible
24 hrs	Not Detectible
72 hrs	Not Detectible

susceptible volunteers, we can not recover the virus from them. The volunteeers did not develop clinical rubella and there was no evidence of transmission to other susceptibles in the population.

DR. MEYER: Thank you, Dr. Sever. Doctor Hok, we only have about 10 minutes. Do you think you can get yours in?

DR. HOK: Yes.

The slides show the figures on inactivation of rubella virus grown in African Green and by and large as you can see the inactivation progress is quite rapid and appears to be comparable to the curve that Dr. Sever just showed a few minutes ago.

I might add that this rate of inactivation was reproduced several times and also the point at which virus was no longer detectable varied from two to eight hours in the different experiments.

The only other comment I might make is that the material treated for eight hours, 24 hours, and 72 hours was tested rather exhaustively for residual virus, not only in primary African Green kidneys, but also in RK-13 cells, in BS-C-1 cells and in Althausen T-2 cells , passage twice and back-titrated in primary African Green kidneys challenged at all these points, and we have been unable to detect any live virus after treatment for eight hours.

This just about summarizes what I have to say on inactivation. It was 1 to 4,000 formalin.

DR. ARTENSTEIN: Was it given to any humans?

DR. HOK: No, it was purely laboratory.

DR. MEYER: Anyone else have any experiences they would care to impart on formalin inactivation of rubella virus?

DR. ARTENSTEIN: I would like to ask Dr. Sever a question. Following the administration of the inactivated virus vaccine, what is to date the longest duration of antibody persistence, to date?

DR. SEVER: The only data we have available as of this point is that which we have just shown.

DR. ARTENSTEIN: How long was that interval following vaccination?

DR. SEVER: I think 38 days was the longest after the vaccination. The type of information of course which is needed is exactly what you are referring to, and yet the problem of doing these types of studies are large as I am sure you know.

DR. ARTENSTEIN: The reason I brought that up was because if it were at least three or four months, one might consider this type of vaccine for use the first day the woman reports to her obstetrician and is diagnosed as being pregnant.

DR. MEYER: Dr. Buescher, do you have some comment?

DR. BUESCHER: No. I would like to ask Dr. Hok whether his preparations are antigenic for rabbits or any laboratory animals?

DR. HOK: Yes, we did have antigenicity on material inactivated for 24 hours. I might add material inactivated for ten days was no longer antigenic for rabbits.

DR. BUESCHER: And the starting material is what? To what do you add the formaldehyde?

DR. HOK: The starting material is virus grown in African Green kidney cells and serum-free media.

DR. BUESCHER: The whole works?

DR. HOK: No, the gemische that has been clarified.

DR. WARREN: I would like to ask Dr. Sever if he plans to challenge any of his vaccinees with natural virus, i.e. serum instead of monkey kidney?

DR. SEVER: Yes.

DR. MEYER: Doctor Parkman?

DR. PARKMAN: I just wanted to comment that we have done some formalin inactivations of fifth passage grivet monkey kidney tissue culture fluid and our inactivation curves correspond quite well to those presented by the other investigators. However, with material inactivated at 37 degrees for 24 hours, we have not been able to show antigenicity

for monkeys, either monkeys or guinea pigs, and the monkeys at any rate are not protected by three, 1ml immunizations, therefore our results are at variance with the other people. I just mention them here for that reason.

DR. WELLER: I would like to ask about the starting material Dr. Sever used. It has certainly not been our experience that you can get an antibody titer of 1 to 512 on rabbits on the basis of one inoculation of live virus. That is one point I would like clarification on.

The second point has to do with whether any studies were done on the decay of formalin during the inactivation period and whether there was a residual of formalin left at the end of inactivation, used as a safety factor, and then I think we ought to think more about the fact that in that particular series, apparently the antibody titers achieved as a result of vaccination were the same as those achieved as a result of challenge, which all points up to me that there is something still live, it may be modified, but it looks as if it replicates.

DR. SEVER: Starting with the last part first, this has been our major concern, of course, and we have worked very extensively, our group have commented on this a number of times, that the presence of undetected modified undetectable live virus is a very real consideration. We have not been able to detect it and we are continuing to use

all available techniques to recognize the presence of this material. In any event, clinical rubella was not produced and the virus could not be re-isolated from the individuals who were given this material.

Concerning the decay of formalin and serum, we added bisulfite in the usual procedures and as I am sure you know, Dr. Weller, the problem of measuring formaldehyde, at least in our hands, is great. I think most laboratories have some difficulty measuring formaldehyde levels. Tests are being done rather extensively, biochemical tests are being done on this questionof decreasing amounts of formaldehyde, the effect of protein in the media on formaldehyde inactivation, and the presence or absence of residual formaldehyde after bisulfite.

The starting material for all of our rabbit studies and most of our hyper-immune material is tissue passage, 524 passage, in African Green rubella virus of the RV strain, which Dr. Schiff mentioned before, and has a titer of approximately 3 logs, when measured by the interference method. Titers then are determined by the interference method, using a half log of rubella virus.

I know that a number of other investigators who are here have titered rabbits and I wonder if they would care to comment on their findings with similarly prepared serum?

VOICE: : I would like to hear a comment on that point as to how easy it is to make high titer after 1 or 2 injections.

DR. ROZEE: The highest titer serum we get with rabbit inoculation is 1:64.

DR. PLOTKIN: Our results are, in rabbits given live virus, running between 1 and 16, some 1 and 64, occasionally 1 and 128.

DR. PARKMAN: Our experience with repeated, in our experience repeated immunization of rabbits in our hands, will yield sera which have quite satisfactory titers.

Now the absolute titer level varies with the virus dose used in the test. So that to give the titer is not really a good, just say the titer is 1 to 512 or 1 to 16 is not sufficient. Against a virus dose of 100 interfering doses, the same serum may have a titer of 1 to 8 and against 10 interfering doses it may have a titer of 64 to 128. That is about the range of titers that we have observed with our rabbits. And generally to get high titered sera and by high titered, I mean 1 to 128, versus 30 roughly interfering doses, have required in our hands repeated immunization with live material.

DR. BUESCHER: One of the points that may bear on this are thd differences in the slopes of the curves of neutralization that Dr. Parkman showed yesterday and that

Dr. Sever showed this morning. I didn't have a long enough look at the slide this morning, perhaps we can compare these this afternoon, but our ratio, the ratio we ordinarily obtain is a four-fold reduction for each ten-fold increase. It looked to me from this slide as though there were eight to ten-fold. It was more of a 1 to 1 basis; in other words, the slope of the curves were totally different. The significance of this at the moment I can't possibly evaluate. But I think it would be worthwhile this afternoon to get those slides back and compare them.

DR. MEYER: Dr. Rosee, we have time for one quick comment.

DR. ROSEE: I want to clarify what Dr. Parkman says is quite correct, this wasn't a single injection, ,this is a series of injections at usually four-day intervals of material that measures between 3.5 and 4 tissue culture doses given one ml intravenously and one ml intraperitoneally, and this is the titer we usually get, 1 to 64, sometimes higher, sometimes lower, 1 to 64 is the routine.

DR. SEVER: I believe it is now clear the test is more significant than the sera we are talking about. This has occurred every time we talk about titers. How much virus you use in the test is undoubtedly the most significant thing. Dr. Parkman's data yesterday, Dr. Schiff's data that we referred to again and perhaps, I don't have a copy of

that issue of Science, but I understand the slides have already gone upstairs, so I can't get out that slide right now, but as Doctor Buescher suggests, it would be of interest to see how close these slopes are.

As I remember, your slope though, has logs of sera.

DR. PARKMAN: Logs of antibody and logs of virus dose.

DR. SEVER: Ours has logs of titer, so it would be a semi-log compared to a log log comparison.

DR. MEYER: Before we adjourn, there has been this question kicked around, the possibility of live virus in formalinized vaccine as a general question. I hope somebody who has access to people -- as regards monkeys we have titration results on sensitivity of tissue grown material in monkeys, we know how this compares to tissue culture titer. I hope someone who does the clinical trials takes some of their tissue culture material and does a titration. This would be interesting results to get. It would be the first time you had some idea of tissue culture in man as compared to animals.

Dr. Krugman has this on fresh material, human serum, and this is entirely different from tissue culture material. This is true with measles and probably true with rubella. We have run fairly late. We will adjourn now until two o'clock and finish up this afternoon.

(LUNCH RECESS)

AFTERNOON SESSION (2:00 p.m.)

DR. MURRAY: (Presiding) Can we take our places, please. I think it will be generally agreed that the presentations which have been made yesterday and this morning give indication of a tremendous amount of advancement since the initial isolation of rubella virus as something we could work with.

The main purpose we had in mind in calling this meeting was to give people an opportunity to discuss these things, but against the background that information of this kind is among other things designed to lead to the eventual development of vaccines and other agents for controlling rubella.

As I explained yesterday, it will be necessary before a vaccine or similar preparation can be licensed, that regulations should be in effect and that these should be realistic regulations based upon experience, so that when the vaccine is ready for general distribution there will be no delay in the licensing of the product as a result of just inability to formulate standards.

A great help in this matter would be the development of reference preparations, perhaps not standard preparations yet, but certainly reference preparations which could be distributed and used by investigators in the field as they wish, so that their own methodology could be tied in to a common preparation.

In the past we have generally aimed at, in the case of a vaccine of this kind, having a serum of some sort, usually from one lot, but which had been specially investigated from the point of view of its antibody content and this was circulated to investigators for their use and when it was possible to secure a reference virus preparation, this could also be circulated for standardization purposes and using these references then it would be possible to define what the variables are in the test systems and also when it came up to the point of licensing the data which had been accumulated in diverse laboratories could be tied into one another by the use of these materials.

This is what we would hope for in the long run. There are a number of questions we would like to throw open for discussion this afternoon and I hope the discussion will be free and uninhibited because the whole area is in a state of fluidity at the present time and this might be different in a year or two when a certain amount of rigidity has been introduced by the use of particular methods.

We had hoped we could discuss such things as questions which relate to the virus, rubella virus strains, the evidence for homogeneity, or are there other strains, immunogenicity, and such matters as whether there is any relation to other viruses, the question of oncogenicity, and so on.

Then questions relating to the immunity in rubella. Many of these things have been discussed during the past two days. Antibody response and persistence of antibody after infection and after vaccination will be most important things in relation to immunization and the relation of neutralization antibody to immunity against either the clinical rubella or the teratogenic effects.

Also important are such matters as the potential cell culture systems that are available and questions relating to the development of controlled tests.

We are also faced with the problem as we approach vaccine development of a suitable vaccine strain, its history, the method by which it was isolated, and the systems in which it has been cultivated.

Too often in the past the isolations have been made in systems which later proved to be inacceptable and new isolations have had to be made and it is not certain how the data with the new strains could be compared with those obtained in the older systems. That has certainly been the case with some of the earlier isolations of adenovirus. And we have also seen this with influenza.

Now I would like to turn some of these items over for general discussion and perhaps I could follow along a list of items which I have here which I mentioned.

First of all there are the questions relating to

the rubella virus strains, evidence for antigenic homogeneity.

Does anyone have any ideas or theories on this? Are we dealing with one strain? Or are there more than one? And does anybody have any information about the work of Norrby and others in Europe?

DR. SEVER: I would be willing to contribute our information concerning strain. We have worked with isolates at at least eight different locations now and cross-checked these with hyper-immune specific anti-sera and paired sera from patients with the disease and found no serological difference between any of the isolates from any of these locations. These locations are primarily from the United States, Alaska, and several island populations.

Outside of Dr. Norrby's work on electron microscopy with rubella virus, I don't have any other information in that connection.

DR. PARKMAN: I only have the data which I presented in a discussion of the properties of the virus. We had studied viruses from 1960 to 1962 from a variety of areas in the United States and one isolate which was made from a patient who acquired rubella in Germany, and these viruses were all equally antigenic in rabbits immunized with identical immunization schedules, and as I showed on the slide the cross-neutralization tests suggested they were antigenically homogeneous.

DR. WELLER: You really raise two points. I think it is premature for us probably to attempt to be dogmatic about whether there is a hetereogeneity among strains until we refine our serological techniques. At the moment I think none of us can show differences but until we get better reference sera, better ways for running our tests, I think it would be quite rash to assume that we are dealing with just one antigenic type agent and there are no variations whatsoever.

The other point you raise, about Dr. Norrby'S work in Gard's laboratory, I think deserves some discussion because I would like to hear if there is any confirmation of Norrby's conclusions that rubella virus morphologically seems to be similar to murine leukemia virus. If this is confirmable, in this country, then I think we have to realize we are dealing with an agent quite different from agents we have worked with before, and that we may have to develop new ground rules in terms of safety tests, precautions, and if it is true I, for one, would be very hesitant to put tissue culture material into human volunteers until we know a little bit more about what we are playing with.

So I would like to ask this group how about the electron microscopic findings in this country? Do they or do they not comfirm Norrby's report?

DR. MURRAY: Has anybody done any electron

microscopy with rubella?

DR. VERONELLI: We have had some, many months ago, something like a membrane in shadowed preparations of supernatant fluid.

DR. WELLER: Have you seen the Norrby paper? Did they resemble that?

DR. VERONELLI: It could be thought under the different methods of preparation, that his work and ours were comparable.

DR. ROBBINS: How did you get your preparation?

DR. VERONELLI: Supernatant fluid, concentrated by carbowax, , as indicated.

DR. MURRAY: Thank you.

It would seem in view of this --

DR. ROBBINS: I think a good many people could give negative data.

DR. MURRAY: It seems to be something we should obtain some information on.

DR. ROBBINS: If we could take up the other topic that you mentioned, if one assumes that -- I have been kind of impressed at the fact that most of the systems we are using for determining antibody don't seem to give you very wide spread, and if this is so, we are dealing with quite an insensitive system, regardless of what kind of slopes you show here, then this would mean we would not pick up

degrees of variation in antigenicity that might be of significance in regard to immunity and so on. So that I think that Doctor Weller's point is very well taken, that until we have more sensitive methods of comparing antigenicity, that we don't really know. We only know that they belong to the same family or group, or they have relationships, but we do not know they, how closely related they really are.

Would the immunologists in the crowd agree with this?

DR. FELDMAN: Could we look at it another way? Assuming this virus is a member of this family, produces epidemics every 10 years, then if these were significantly different from one another, one might expect in successive epidemics there would be different groups or multiple or recurring group attacks. Actually has anyone observed that let's say those at the current time who are 11 to 15 years of age having any more or as much disease as those 10 or under or those over 40 or between 40 and 50, having epidemics with equal frequency, or being attacked as often as those under 10?

If this were different, there ought to be differences. You see this with flu, but not in my experience in rubella. I think this would be a potent argument against it.

DR. BUESCHER: I think a great deal of ignorance in this matter stems from the fact that this is not a

universally reportable disease. I should think one approach that might be undertaken by the Institutes of Health here is, one, to see what can be done about getting this disease reported, fully recognizing that what is going to be reported is going to be a hodge-podge.

DR. MURRAY: Doctor Weller?

DR. WELLER: I have great respect for arm chair epidemiology, but I still like the laboratory. I wonder if we could have predicted in an arm chair that there were three polio types on the basis of the epidemilogical data we had in 1940 say?

DR. MURRAY: Dr. Henderson, is there any move to make rubella a reportable disease?

DR. HENDERSON: No. We have not given much thought to making it a reportable disease. I think there has been a great many questions raised at this meeting as to what a reported diagnosis of rubella really represents. There have been some data presented which show that people currently have a good memory for having had the disease, at least it corresponds to some degree and we have other studies which show it has absolutely no correlation with the occurrence of the disease. I am not sure how much we will learn from having it as a reportable disease nationally. It is reported in a number of states now. I think 20-odd do have it reported and we can get the information from these

states on weekly or monthly bases and there we intend to and will be following up on our previous reports on this probably in a month or so. And we had not thought of getting it nationally, however.

DR. BUESCHER: Perhaps the 20 states is enough for the time being. I was not aware there were that many.

DR. HENDERSON: I will say also we are doing a couple of studies, three to be exact, based really on clinical diagnosis, laboratory support being a problem on this, to age specific attack rates which will be an index with limitations attached, which we all appreciate.

DR. ROBBINS: I wonder if we could, since it perhaps is impractical to make it a reportable disease nationally, if it might not be possible however to make rubella in pregnant women a reportable disease? And this perhaps one could get more sympathy for from health departments and this would present the health departments with a smaller load of patients to follow up on and would allow identifying a sizeable proportion of the women who might be expected tohave difficulty. This would be perhaps the key group to zero in on. Is this a feasible thing to do?

DR. HENDERSON: The basic problem in reporting or trying to initiate reporting of any disease is really this information must reach the local practicitioner and he must get into the habit of reporting it in some way. We

found in initiating any sort of reporting it takes several years really before you begin to get appreciable reporting and begin to get some estimate of how well it is reported. But it is rather poorly reported for several years.

It might be possible to work something on this through the obstetrical group or the general practicitioner group.

DR. ROBBINS: I can think of several groups that would be helpful in this. Somebody has to start it. The public would help, I think, in this considerably.

DR. MURRAY: Perhaps some states or cities might want to initiate this on a trial basis initially, not knowing the intricacies of initiating a reporting program, but knowing that it is difficult, particularly looking back at the reporting of hepatitis when this became mandatory, I could foresee some pretty tricky legal problems involved, particularly about confining it to reporting of rubella in pregnant women.

DR. ROBBINS: What in the world could be legal there?

DR. MURRAY: That this is a personal matter.

DR. HORSTMANN: I would like to ask how widely the practice of reporting congenital defects is in this country? I know in Connecticut this is a reportable disease, any congenital defect. Is this widespread or is this just a local thing?

DR. HENDERSON: There are 30 states I believe that have on the birth certificate a spot for checking congenital malformations. This of course is only what is detected immediately. And this is being currently analyzed and studied by a group in the dental research institute, and it is a little hard to define it, but they are doing a very good job on this and getting the information fairly currently. I talked with them just last week about getting data with respect to this and I think they will be getting rather good data on specific deformities which can be identified at birth. But that would cover 30 states and I believe most of the most populous states.

DR. MURRAY: Dr. Krugman?

DR. KRUGMAN: Doctor Weller raised the question previously, he asked would it have been possible to predict that there were three types of polio virus, and of course the answer to this question is no, and the reason the answer is probably no is because the majority of cases are sub-clinical and paralytic disease is a rarity.

On the other hand if this question were raised about measles, the answer based on clinical and epidemiological observations I think would be clearer and that is that one attack of measles in a vast majority of instances with very very rare exceptions is followed by permanent immunity. And it is my opinion that we have enough data

available for rubella. The vast majority of cases of rubella are associated with rash, and it would seem to me on the basis of clinical as well as epidemiological grounds one could even now predict that in all probability one attack of rubella will give permanent immunity.

DR. FELDMAN: There are many problems inthe reporting business. For example, in Syracuse last summer there were many pediatricians and general practicitioners and what-not who were quite convinced we had a rubella epidemic. This turned out to be Coxsackie A_9 with a rash in young children, that is predominantly on the face, and so on. When we ran into an epidemic of rubella, there was no question, some months later.

I think if one wanted to set up a reporting scheme the easiest place to do it is by selecting a group of college or student health services. Our first cases in Syracuse came exactly two weeks after the return of students from Christmas Holidays and this happened at the university student health service, I think there were seven cases one day, and then on up the scale.

One can get the telegraphic reports from a selected number, 10, 20, 30 or 40 or what-not. And I am sure this has happened on colleges around the country. But if you put it on a national reporting basis, I think you will find the prevalence of this disease will decrease markedly in a brief period of time.

DR. MURRAY: Doctor Sever?

DR. SEVER: Being somewhat familiar with the student health service at Northwestern University, I wonder, Doctor Feldman, if you feel the occurrence of rubella in college age students would be a good index, though, of rubella in the community? We have seen at Northwestern a number of outbreaks of rubella which did not seem to coincide with rubella in the community at large and may possibly reflect the susceptibility of that group and the conditions under which it is brought together, as you point out, periodically.

I wonder also if Doctor Miller would care to comment, and Doctor Buescher, on the military population as reflecting the occurrence of rubella in the general population, as an index therefore of the rubella susceptibility?

DR. BUESCHER: Well, I think that in many respects the college student population and these recruits are similar, in that in recruit training centers, rubella is endemic from about the first of December until sometime the end of March. It is there every year. Now I don't know what this means. I think what Dr. Miller said earlier, the likelihood that these incoming recruits are bringing rubella virus with them, I think this likelihood is good. We can state categorically that all you get out of such reporting is quantitative information unless you broke it down and made it a very difficult thing to say okay, we want those cases

that occur within two weeks after they strike the Post and where did these people come from.

I know, or at least I think it is predictable what you will get. You will get the first of December until the end of March, and it will be that way every year.

DR. MURRAY: Dr. Miller?

DR. MILLER: I think that if a real conscientious effort were made at recruit training centers, you could get some pretty good ideas of what was going on in various parts of the country by observing the number of cases occurring a short time after arrival. We have seen this. We have known that rubella was going on in the northeastern part of the country I think a little ahead of the local communities. But I don't know if you could get people to do this in the fashion that would be required to give you really valid information unless you had some research group interested in the problem who kept their finger on it all of the time.

DR. KRUGMAN: As I indicated yesterday, rubella has been a reportable disease in New York City for many years. I agree with everything that has been stated about the difficulty in proving a diagnosis of rubella and many of the cases undoubtedly that are reported as rubella may not truly be that disease, but as one looks at this list, and I have it before me now, from the year 1940 up to the present time, you do get a rather significant indication of

what is going on. Each year the average number of cases per annum has been somewhere between roughly 2,000 and 4,000 reported cases. When in the first two or three months of 1964 there had been more than 6,000 reported cases within the first part, between January and March 11 there were 6,621 reported cases in this short period of time, it became apparent, even with this crude measure possibly of an accurate diagnosis, that an epidemic was occurring.

Now in spite of the fact that the diagnosis is difficult to make, I do think that rubella should be a reportable disease, and that it would give a great deal of information. I queried a number of physicians on our staff during this present epidemic and these are pediatricians in private practice, to try to determine how many cases they had seen and how many cases they had reported. I am ashamed to tell you that a very small percent of the cases were reported. If I had to guess, I would say the ratio of those reported, the ratio is about 10 to 1. There are 10 cases not reported for every case that was reported.

DR. MURRAY: Doctor Henderson I think could confirm this because in connection with measles, CDC, I think, carried out some surveys to try and compare the actual cases with the reported and came up with a figure of approximately 10. And the number of reported cases should be multiplied by 10 in order to arrive at the estimate of the

actual number of cases. Is that right?

DR. HENDERSON: Yes, I think this is right. And I would say that in reporting of certain of these diseases, such as measles, and I think truly with rubella, we are not really, I don't think we could ever hope to get a total reporting. Many of these people don't see physicians, for example. But it will give us an order of magnitude.

As you point out, we would have some idea of when it occurred, and I think that is good. Right now we have some index from the various states and I think this is something we can take up with the state epidemiologists this month, the committee is meeting this month, and I think we might bring this up as a possibility of instituting this on a nationwide basis. I don't think in terms of accuracy we are going to do any better selecting out any otherpopulation group in all fairness to the military, even here, where you are seeing many of the recruits there are a fair number of errors in diagnosis and we have had an opportunity recently to review a great many charts with relation to encephalitis and measles in military populations. We got some idea of measles encephalitis in adults and we find actually many of the diagnoses are very probably in error and we are left with a bit of a pot porri, even in this group, and this I think is understandable. I think it wold be the same in a college population. And I would favor the idea, really, of

maintaining a broad reporting system simply as an index without fooling ourselves that this is in any way truly representative of the incidence in the country.

DR. PLOTKIN: With reference to Doctor Robbins' suggestion concerning reporting in pregnant women, based on our experience in Philadelphia, I would suggest two handles by means of which these data could be obtained.

One, in Philadelpn ia, at least, gamma globulin is given out by the City and consequently every physician who wants some for his pregnant women exposed to rubella must report the disease.

Secondly, most hospitals, all accredited hospitals, have therapeutic abortion committees, consequently I think it would be possible to determine the number of abhortions being done for the actual incidence of the disease.

DR. MURRAY: We could spend a lot of time discussing these matters, but could we move now to some of the other items that we have under consideration. For instance, the immunogenicity of different materials that have been examined. Are there any differences, at different passage levels, and how about the communicability from person to person? Some evidence has been brought forward in the last few days on this, but this certainly is not a great deal to base any policy decisions on. Could we have some thoughts expressed on these matters?

DR. MEYER: I think everybody has about the same thoughts on the matter, and that is that insofar as communicability, we have already mentioned it several times, there is a little bit of data in man, not talking about the raw material, but talking about tissue culture material, there is very little data in man, there is a little bit of data in monkeys, but not very much data and I think the only thing one can say is this is a subject that obviously people will be interested in and I hope those who plan experiments plan them in such a way, if they are doing further experiments with animals, that they do have cage controls, room controls, and serology in these animals, so as much experience can be accumulated in this field as we can get in the coming months.

DR. MURRAY: Well, it has occured to us, as I am sure it has occured to most other people, that if consideration is to be given to a live vaccine at some time this question of communicability is going to be a very crucial one. In the case of measles, this didn't turn out to be much of a problem, fortunately. But the crucial experiments that would have to be done here I don't think people are going to undertake except by accident.

VOICE: Could you clarify that point?

DR. MURRAY: Well, if the danger is to pregnant women --

VOICE: How about under controlled conditions in

an institution where you have isolation units, and no pregnant women?

DR. MURRAY: Well, that is one thing. But there you are restricting the use of such a vaccine to something other than universal use.

VOICE: This is for trial purposes I am talking about.

DR. MURRAY: Yes.

DR. SEVER: I agree completely that we need considerably more information about the problem of communicability. I think it is quite clear that there are relatively few places where one would wish to conduct studies using live virus given to children, volunteer populations. Really relatively few locations where safe isolation facilities are available and where the attendants themselves are not likely to transmit this virus which you have artificially introduced back intothe civilian population. So that as these studies on communicability with either killed or supposedly attenuated materials are designed, they will have to take into consideration these rather rigid requirements concerning the facility and the population which one can use.

Of course since we are talking about the most significant transmission being to young adult females, we must consider that the data you obtain for one population group may not be applicable to another group if you are dealing

with different ages or sexes. And the problem of dealing with a group which you really want to determine is it communicable to is the one which you probably, as Doctor Murray suggested, would not wish to include in your study design.

DR. MURRAY: Doctor Krugman is right, it can be done, but it is not going to be easy.

DR. KRUGMAN: It was done for measles in this way, but initially under controlled conditions and this is where some of the original data were acquired.

DR. MURRAY: For those who have been working with strains at different passage levels, is there any indication that there is any change in the immunogenicity with passage level? Perhaps we are getting too far ahead of ourselves on this, and that is we should have been discussing perhaps methods for determining antibody levels, are they sensitive enough to enable us to make any deductions in this respect at the present time?

DR. PARKMAN: The only data we have about this is we have immunized rabbits, again with live virus, and immunizing one series of rabbits with virus which was higher than 10th passage, and unfortunately the other series of rabbits immunized with low passage virus were not the same virus strains. However, the anitbody responses in these animals were comparable. So at least with live virus innoculated into rabbits,

there doesn't seem to be an attenuation of the antibody producing ability.

DR. MEYER: I think you can probably say, this is jumping at conclusions a bit, but at least from the data, limited though they are, that have been presented both in relation to human infection and monkey infection, that insofar as you can tell the antibody response to live virus seems to be as in measles, an all or none phenomenon, you get the impression that either children or monkeys, either get infected and get antibody titers in some general range or they don't get infected.

Certainly there were several ranges of monkey dosage used in some of the experiments you did, and the antibody titers were in the same range. And I think this has been roughly true of the human experience too. But the information is still rather spotty.

DR. BUESCHER: I would like to ask a question of the group. If there is concern concerning possible variation in antigenic structure, would an experiment in which a number of Rhesus monkeys, small numbers in each group, are infected with a series of strains, recovered from different places at different times, and cross-challenged, would such an experiment be satisfactory to Doctor Murray? And if you could show that infection of monkeys with "x" number of strains protects rather uniformly or entirely against the same number of

cross-challenges, would this be satisfactory? This is the kind of experiment that can be done and doesn't require people to do it.

DR. MURRAY: Well, I think obviously it would be foolish to give a definitive answer to a question like that. All one could say is I think such an experiment would be valuable, as indicating that there is cross-protection. And even if there were some antigenic differences in the strains, that you might get cross-protection. This is the kind of exercise we go through with minor changes in influenza virus. I haven't been asked to give the answers to the questions, we were trying to get some answers from the audience.

DR. BUESCHER: I was asking the group.

DR. MEYER: I don't think this would be necessary. I mean just as a guess. I think Dr. Krugman was pushing epidemiological data. I think there are so many sources of good epidemiological data, the various island populations, . there are a number of ways you can go at this. I can't believe with the type of epidemiologcal data you can get if you go looking for it and with the types of laboratory studies you could get, such as Dr. Weller mentioned, when you think of conventional cross-neutralization tests and this sort of thing, I think it would be unusual if you couldn't resolve the point beyond reasonable doubt by that type of approach.

DR. MURRAY: Doctor Cabasso's presentation this

morning on neurovirulence brought up some matters which we thought might be worthy of discussion, and that is, although the findings were negative, is this a matter which should be pursued, knowing that the test that was conducted here was specifically designed for polio virus? And that the timing and the changes that were looked for depended upon the properties of polio. Now should such tests be done, but continued over longer periods, or lesser periods?

DR. CABASSO: There is also the question about different isolates and different passage levels. This was one particular isolate on the 25th passage level. So I think other isolates, other passage levels should also be tried.

DR. MURRAY: Now we do have primary isolations available. Perhaps this aspect should be studied with very low passage level material. Any thoughts on this?

DR. CURNEN: Have any electroencephalographic studies been done in human volunteers? Would this be of any help?

DR. MURRAY: The question was have any electro-encephalographic studies been done in humans.

VOICE: You mean experimental infections? There are many reports in epidemics.

DR. CURNEN: Experimental.

DR. KRUGMAN: Do you know of any in epidemics?

DR. MURRAY: Gentlemen, we can't hear you. Well, they are talking to themselves. Doctor Weller?

DR. WELLER: I think we are talking about the same thing, I think Pampiglione published one or two papers on EEGs, perhaps complicated German measles, usually.

DR. MEYER: Didn't that original, one of Gibbs' original pieces of work, where he was comparing the incidence of natural measles with other childhood diseases? I believe German measles was included in that report and I think he reported some instances of encephalographic abnormality. I believe I am right.

DR. MURRAY: Well, if such changes were noted, then certainly it would almost be incumbent upon us to obtain similar information on infection with the passage level comparable to what would be used in a vaccine, if any such work was to be done. I think this would have to be, at least it would be our view that this should be done, despite the fact that there was some discussion as to the meaningfulness of this work as far as measles was concerned.

DR. SEVER: Doctor Murray, do you mean now in connection with the animal testing, monkeys, that preliminary studies of the monkeys should include electroencephalograms?

DR. MURRAY: No, we are not talking about that. We are talking about the administration of rubella virus to, experimentally, to human subjects. I don't know what a monkey encephalogram would show.

DR. MEYER: Doctor Murray was referring to --

DR KRUGMAN: Doctor Cabasso just handed me a reprint of Dr. Pampigiione's report and I think it would be worthwhile reading very briefly. It is only three or four sentences from the summary.

"Seven patients with mild and three with severe forms of encephalitis during the 1962 epidemic of rubella are reported. All patients but one made a full clinical recovery, although mild EEG abnormalities persisted in most of them. Uncomplicated cases of rubella did not usually show eeg abnormalities in contrast with measles, either at the time of the rash or later in patients without pre-existing cerebral trouble."

DR. MURRAY: Well, those are questions concerning strains and matters which would be of interest to us about immunity and I think would be of very great pertinence as far as the eventual development of standards for a vaccine are concerned.

We do need information and we have some of it, about the persistence of antibodies after natural infection. I think many of us were perhaps a little disappointed to see how quickly they go down. But I don't know, perhaps we could have some discussion about this.

Dr. Krugman?

DR. KRUGMAN: I think Dr. Green could probably comment about that.

DR. GREEN: Well, the slide that I showed of 6 out of 9 subjects who admittedly were followed for a relatively short period of time indicated that the levels, peak levels, were reached around a month after the rash occurred, and that they were maintained at peak levels for at least six to 12 months, and we haven't followed them longer than that. In a very small number of subjects, of course.

DR. MURRAY: Does anybody else have information? Dr. Sever?

DR. SEVER: I was not familiar that the data was presented in these meetings showing this loss of antibody with time.

DR. MURRAY: I was just making a provocative statement.

DR. SEVER: I think most of us who have reported have shown a persistence of antibody with time, or within the sensitivity of the tests which were available. Except I think Dr. Rozee, who is not here unfortunately, he had something about the drop or change in the titers among older age groups.

DR. PARKMAN: We have some data we measured in our veterans, who ranged in age from 60 to 80 years old. We tested their sera along with a group of sera drawn from military recruits, who were 17 to 21 years old, and found that the geometric mean titers of those sera which contained antibody were about between 2 and four fold lower for the

veterans, for the elderly men. Detectable antibody was present in 16 of the 18 of these sera, however.

DR. KRUGMAN: Where did they live and were they in contact with children and other personnel that may have acquired rubella?

DR. PARKMAN: These sera came from a veterans home, and I don't know the extent of their contact with children, but I would presume it would be very slight.

DR. MURRAY: But we don't have very much information on artificially induced immunity yet.

Another question which would be of importance I think and which I will throw out for discussion although some information may have been presented on this is the relation of the antibody level to the development of clinical rubella. Dr. Krugman?

DR. KRUGMAN: Dr. Green presented that paper.

DR. GREEN: Well, I think it was the first slide I showed, and of 37 subjects who had sera antibody in the dilution of 1 to 4 or were uniformly resistent to infection, whether by inoculation by virus or by contact.

DR. ALFORD: I think the problem is probably the negative antibody, perhaps with these varying doses it might be quite difficult to decide exactly who is susceptible and how much virus would be, would have to be neutralized in one of these systems. For instance, the half log of virus

that is being neutralized, perhaps the negatives there would be more pertinent. You want to pick up the susceptible population with this, not the ones who had it.

DR. BUESCHER: I think our own experience has shown that with limited populations, it is possible to predict with reasonable accuracy who is susceptible. However, I don't think that the status of the art is such that this could be done on any large scale. This is what the problem is. I think it is possible to decide, and the tests that Doctor Parkman has worked out rather convincingly I think show that under those circumstances no antibody at 1 to 2 is 99 or 98 percent synonymous with susceptibility in a military situation to infection.

Conversely, antibody present at 1 to 2 has a comparable kind of specificity for resistance.

DR. KRUGMAN: D ctor Green showed previously the attack rate was 85 percent in the group that Bob I believe had less than 1 to 4. Is that right?

DR. GREEN: Yes.

DR. KRUGMAN: Just to fortify your statement, Dr. Buescher.

DR. MURRAY: Well then perhaps with the improvement or refinement of methods of antibody measurement, which may hopefully occur, these things could be further defined and re-defined.

DR. MEYER: Just pointing out one deficiency that is obvious to everyone and that is the information that has all been given has indicated that I think even though neutralization tests vary, at least the evidence is consistent that the presence of antibody prevents clinical disease. If that is naturally acquired antibody, except for the small bit of data given by Dr. Sever, there is very little evidence as to what artificially acquired antibody does in preventing clinical disease, either artificially acquired by inactivated antigens or tissue culture material. This is something to be found out.

DR. BALSAMO: We have infected some individuals with tissue culture material. On re-challenging these patients, by putting them into contact with patients who had the naturally acquired disease, they resisted infection and there was no change in their antibody status.

I should mention also that the virus isolated from patients infected with tissue culture material was inactiviated by hyperimmune rabbit serum made from a different tissue culture passage than the one that was administrered to the susceptible patients. And the serum from the patients who contacted tissue culture disease, the convalescent serum, neutralized rubella virus made from a different passage.

DR. MURRAY: Doctor Robbins?

DR. ROBBINS: I am perfectly willing toaccept

the relationship of serologic immunity by any of the tests that we have heard presented here, and resistance to disease, or insusceptibility to disease.

I think the more important question and the one that is going to be very difficult to settle, if we ever can, is whether or not this does completely protect the fetus. And this of course is a spectre that haunts us all. The data on this are many of them anecdotal, and start with such as Schick's diaplacental disease and so on, the rather small difference presented by Lundstrom in his figures, yet they are just enough that they make you worry a bit. This may relate to loss of antibody down to levels which are not protective, because nobody has had prior antibody determinations, so that they know what was the status of that mother's serologic immunity.

In thinking about how one might tackle this, one way of course is the large epidemiologic approach, such as Lundstrom used, which again always has the fallacy in that you have to rely, unless you do a tremendous amount of serology, you have to rely on the past history, which isn't too reliable, and the other is if we can get a suitable animal model, in which this can be studied. And whether the rabbit is going to prove to be this, I don't know, or the monkey. I know Dr. Sever so far has had, I gather, poor luck in inducing anomalies in pregnant monkeys. But we

do have evidence that the monkey sustains the viremia, and this gives me some degree of hope that perhaps further efforts in this regard may yield some useful results.

It certainly should be tried. Otherwise I find it a very difficult problem to answer. Unless we can get specific observations on the offspring of mothers, such as the group in Boston have been able to get, where the mother has been demonstrated to be immune. Yet you can isolate virus in the baby.

I also would like to raise the question as to whether or not the use of leukocytes for isolating virus in the blood might not raise our score a little bit. I was sort of hoping the group from New York would have something to say about this. Hasn't Dr. Gresser some evidence in this regard?

VOICE: I have tested two patients' leukocytes for the presence of virus, from the blood and tested it at the same time with throat washings. In both throat washings, I found the virus, but not virus from leukocytes.

DR. ROBBINS: This was after the rash?

VOICE: Yes, on the first day of rash.

DR. MURRAY: Well, I am glad you brought that up, Doctor Robbins, because that is one of the things we wanted to ask. Would it be improper to decide on this by inference,

that perhaps you can show that women who have a certain antibody level don't have these things happen?

DR. ROBBINS: If you can show that.

DR. KRUGMAN: If we assume that viremia is necessary for fetal damage, I think this is the question of whether we can make this postulate. If we can postulate that in order to have fetal infection you have to have viremia, and if you don't have viremia, in all probability you will not have fetal infection, I say in all probability, because it is impossible to be categorical about it. Now the evidence that has been presented at this meeting would indicate that in the presence of antibody viremia to date has not been detected. And under these circumstances, I would think that it was hopeful and I wouldn't be pessimistic. I do think the studies you referred to previously, Fred, are anecdotal. I don't think they have been proved.

DR. SEVER: I would like to suggest that probably the only way we are going to resolve the question of antibody and protection from fetal defects will be to do the studies which are necessary to elucidate this and this is an opportune year;in our own laboratories we have set up a number of groups where physicians giving gamma globulin because of exposure in the first trimester are obtaining a sera sepcimen prior to the ti e they give gamma globulin, another specimen taken two months later. Doctor Lundstrom has set up a

similar regimen in Sweden. If this were expanded, I think we might be able to gather enough data from multiple sources to determine those who are at risk, certainly those who get inapparent rubella, those who get clinical rubella, and correlate this with the outcome of the pregnancies, assuming the cooperating groups are going to follow those children long enough.

DR. GREEN: We have a similar study underway in New York City from four to six weeks now.

DR. SEVER: The only group lacking are those who don't get gamma globulin, should it prove to be effective or partially effective and there are some medical groups who with the present status of information are withholding gamma globulin and these provide this group for the study of rubella without gamma globulin complicating the picture.

DR. MURRAY: I would like to just throw out the idea that if this question of viremia is going to be one which is of some importance in the ultimate decision of the role of rubella vaccination, that the time to get this information is while everybody is enthusiastic and ready to do this kind of investigation. I can tell you that we all went through a pretty rough time trying to get this kind, these kind of data on live polio vaccine, at a rather late date in the game. It was not a very happy experience for everybody concerned, and I would certainly be delighted to have this

information at an early date. I think this would be a convenient time to stopfor a few minutes.

(Thereupon, a short recess was taken.)

DR. MURRAY: I think if we, despite the thinning out of the ranks, I think if we attend to the remaining items that we have here, perhaps we could conclude the rest of the meeting in about 20 minutes or so.

I think we have discussed most of the important matters, but as far as we are concerned, there is one or rather two rather essential items which we would like some advice on before we get away from here this afternoon.

Perhaps we should turn to those first. The first of these is reference preparation for antibody determinations. We have previously skirted on this earlier and we have two things in mind, one is a gamma globulin preparation and the other is a serum preparation. The kinds of things we have in mind are that we don't know what the future holds in the way of sensitive tests and we have to hedge our bets, I think, not fix on one or the other.

A gamma globulin preparation would be rather stable, we would have this in large volume, and it would be quite useful particularly as it is used for otherpurposes. However, if we should ever get to anything other than the present testing systems, gamma globulin might not work, particularly this is true if we should develop a complement-

fixation test. And we would have to have serum preparation for that.

I would like Dr. Meyer to comment on this matter both of the gamma globulin and perhaps the serum as a prelude to the over-all discussion.

DR. MEYER: I think it is pretty straight forward. It seems to me that the outstanding need right now, which everybody has said, is for some means of comparing serologic results from laboratory to laboratory and at the moment comparing serologic results means comparing neutralization tests, by whatever method is used.

It seems that any stable serum or gamma globulin preparation that has some reasonable antibody titer, as long as everybody tested, would work perfectly well. So for this purpose, as Dr. Murray indicated, we have the NIH reference globulin preparation, we have it in very large quantity, so we can easily make it available to anyone who may care to use it as a central standard for comparing serologic titers.

For example, if Dr. Buescher's group gets a titer of 1 to 60 with it and someone else gets a titer of 1 to 1,000 with it, you can still by doing a little calculation compare what the antibody titer would be on a corrected basis between the two groups.

We have this material, it is in NIH lot 175, and it

is available to anyone who may care to request it. As he mentioned, if other tests become available, such as CF, it might be useful in the future to have some type of human serum, such as human convalescent serum. There are several you might want, but at the moment I don't see there is any particular need for this, until we have more information on the complement fixation test and more information on other procedures.

Now if anyone would like to comment, I would be interested in hearing it. B,,t it seems to me a single preparation of gamma globulin would serve most of our central standard purposes at the moment.

DR. BALSAMO: I would like to mention again I think that NIH 174 has been tested by more people here; we have tested 174, haven't we?

DR. PARKMAN: Yes.

DR. SEVER: I am not sure.

DR. SHIFF: Yes, I believe 174.

DR. MEYER: Doctor, for the purpose of what we are talking about, the reason I mentioned 175, Dr. Parkman, all of our group have tested 173, 174, and 175, and the only reason I specifically mentioned 175 is I am thinking about something that for some distance in the future would be useful. We could use 174 if necessary. There is still a moderate amount of this left. 175 will be the new lot and it is in

much larger quantities. If there is some particular reason why we should use 175, it still is in sufficient quantity to be used, but I thought the titer of the two lots are essentially the same, at least in Dr. Parkman's test it appears to be about the same.

DR. MURRAY: Dr. Kempe?

DR. KEMPE: I would like to make a comment about the other possible use and that is that of prophylaxis in the single exposed first trimester pregnant woman. That is this: I think it has been indicated twice that doctors by and large will use a gamma globulin product, even despite Dr. Krugman's and Dr. Green's results, for sometime to come, even though they have been told or will be told that it is not doing the job at the dose used.

Now the dose is a factor here, because the arbitrary amount of 12 ml in one case and 20 ml in many other cases is arbitrary in the sense that it is physically what you can reasonably get in. But I said before, some people give 40 cc's and that is arbitrary. Since gamma globulin is nothing except what is in it, the dose has been picked arbitrarily, there is still I think a case to be made for making a lot of convalescent serum pooled and a separate lot of convalescent gamma globulin, to try to see if using the optimal antibody content, the maximal dose, there is not a possible effect, if these studies of Dr. Green's are to be

final, that the best product should be used and the best product has not been used.

DR. MURRAY: Well, are you talking about gamma globulin to be used for prophylatic purposes?

DR. KEMPE: Yes.

DR. MURRAY: For clinical investigative purposes?

DR. KEMPE: Yes, and the point is we now have an opportunity this year to get such a lot of 2,000 units quite readily. It would not be a difficult thing to do, I think. I wonder if we should not take advantage of it.

DR. MURRAY: Well, I think that is a separate project, Dr. Kempe. The thing we were talking about is a preparation with which we have had some experience and it is to be used in laboratory tests as a means of correlating them. What you are talking about now is the accumulation of a substantial amount of material from convalescents in the hope it might be demonstrated to have some clinical value.

Any other views on this?

DR. ROBBINS: I think as a bit of perspective, if you will remember the study of McCracken in England -- wait a minute. Well, the study that was done in England, on the prophylactic effects, they did, as the English are wont to do -- it is McDonald, excuse me -- wont to do, they did some calculations and they calculated that assuming an effect by the gamma globulin,and as you will remember, their

effect was predicated on their study of infectiousness of the disease in the household, it was not a direct control, they found that pregnant women exposed in the household had an attack rate of 3.7 percent and their gamma globulin, they had a 2.2 percent, no, a -- it depends upon the dose, 2 or 1.4, depending on the dose. They calculated that 13,000 injections did not protect more than 50 children from rubella defects, assuming an effect. So that even if we had a large amount of gamma globulin, I don't thinkwe are going to solve a problem this way.

DR. MEYER: We have dropped the subject of serologic standards. I think everybody, I assume, since no one is arguing, everyone is agreed it would be nice to have a standard globulin preparation and no one sees a compelling need for any other type of serum, such as human convalescent serum at this moment?

DR. MURRAY: I don't think this is true. Dr. Weller, before he left, asked me to mention this question of serum as he thought very strongly that we should have two preparations, at least aim for two preparations, one a gamma globulin and one a serum, that is, looking towards developments in the future.

DR. MEYER: Right. Well, I wasn't off-setting developments in the future. I am talking about today. Today we have gamma globulin lot 175 ready for distribution and at

some future time it might be reasonable to have a serum. No one disagrees with that.

Turning to what Dr. Robbins said, I feel the way you were expressing yourself, it seems to me that there may be a very real need or it might be useful to have a convalescent lot, but it seems to me the only real good use you could put to this would bestudies like Dr. Green and Dr. Krugman, and that type of thing, where you would have some expectation of getting some decent results and this means you need a lot less globulin.

DR. GREEN: We are in the process of doing this now.

DR. ROBBINS: I wouldn't question the value of striking while the iron is hot and getting what we can get and God knows the ingenious things we may think of, but I don't, myself, think that as a prophylactic measure it offers a great deal of promise, whether you have got convalescent or non-convalescent or what-have-you.

DR. MURRAY: I think although this was not one of the main purposes of this meeting, the record will show that there is a feeling that a great opportunity would be missed if we did not move in the direction of obtaining some convalescent gamma globulin at the present time.

DR. MEYER: I think unless I misunderstand Dr. Sever, his comments earlier, didn't you say you had a lot of

convalescent globulin, you were willing to make available to people for clinical investigation?

DR. SEVER: There is a small lot which we will be happy to make available for laboratory determinations, but there is nothing quantity-wise compared to the amounts necessary to conduct a clinical investigation. And several people are in agreement I think or are in favor of that aspect of going forth and getting that type of material. But we do have a small batch of convalescent gamma globulin which we would be willing to make available and we have been contacted by several groups already this afternoon on this.

DR. MEYER: How much? What do you define as a small batch?

DR. SHIFF: We have given some of this out already. It is about I think 100 cc's left of this.

DR. MURRAY: Oh,well, that is very small. Since by and large industrial lot size is approximately the same, namely, on the basis of 2,000 contributions, could anybody hazard as to how many cc's of gamma globulin a single lot would constitute?

VOICE: About one-twentyfifth of the plasma.

DR. SEVER: It is a 1 to 20 concentration in commercial gamma globulin in this country, and allowing for the amount that is necessary to use for safety testing, if you are considering clinical use, you would need I think we

computed 1,000 units of blood, and it ends up giving you enough for 500 patients, using this 20 cc dosage schedule.

VOICE: You get 10 cc's per pint of gamma globulin.

DR. MURRAY: That would be 200,000 cc's.

DR. SEVER: No.

VOICE: It doesn't always work out that way. We had some where you got closer to five.

DR. MURRAY: All I am trying to get at is would one lot be sufficient or should it be more than that? Let's not waste time on it.

DR. SEVER: To answer the problem in prevention of rubella syndrome defects, which is really the question we are asking, does this prevent rubella syndrome defects, I am sure it would not be. Just the data that we have commented on from Sweden would make that insufficient. But if we are going to accept the prevention of viremia as the end point we are trying to determine with this convalescent gamma globulin, then of course it would be very helpful.

DR. MURRAY: Dr. Krugman?

DR. KRUGMAN: I think it is important to realize that by next year there should be very little rubella during pregnancy, following this epidemic the opportunity for large-scale trials will be, the opportunity will be very limited, and the only significant studies I think that could be carried out would be the small trials that we have referred to

previously. It will be very difficult to get the information which is needed, because of this epidemic.

DR. MURRAY: Dr. Shelokov?

DR. SHELOKOV: When we speak of prevention of viremia, I wonder if some of this can not be discussed as a masking of viremia? I am thinking of the demonstration of viruria, long after viremia is discovered, and presumably the virus that we pick up in the urine or bladder, either propagates in the kidney, which I don't think is likely, or more likely is in some way disassociated with the antibody complex.

I wonder if the same sort of thinking may not apply to how we are preventing viremia, how we are masking it?

DR. MURRAY: Well, I don't know that there is an answer to that, but it has to be considered.

One other item that we would hope for some advice on today is the question of a reference virus preparation. Are we at the stage of development of the art where we could obtain enough virus in sufficient titer and preserve it under stable conditions so that it could be used as a reference preparation for virus titrations? Do we have any thoughts on this?

DR. SEVER: I would suggest that either Dr. Weller or Dr. Beescher's group make such a seed or preparation with the cooperation of DBS, if they are willing to make this

preparation available as a reference material and perhaps also make it available through culture collection.

DR. MEYER: We are talking about two different things here. One is the question of various reference strains, in other words, seed strains that different people could get to compare for various types of laboratory work. I think those are readily available. I think if anyone wanting almost any strain can get them by direct request of our group, DBS, Dr. Sever, Dr. Buescher, Dr. Krugman, anyone working will be happy to make seed strains available.

What Dr. Murray is asking is this question we get into in a slightly more advanced stage of development, and that is, whether it is or is not valuable to have an enormous number of ampules, say several thousand ampules of a particular lot of virus, that really about its only use is as a titrational control in serologic tests. Now is this a necessity at the moment, and I will give my view on it. I dont think it is particularly urgent at the moment, but there may be others who feel that it is. For example, our measles virus potency assays, live measles vaccine, we have a reference material that is sent out to titer as the manufacturer makes vaccine, he titers this material so he can compare the titer of his vaccine with a standard material. This same sort of control can be used on a neutralization test. The question is do we need a standard

of this sort. This means getting to the level of several thousand at least ampules of one particular lot of known titer.

DR. ALFORD: We have a variation from amnion to amnion, even if we use the same batch of virus each time. I wonder if in /African Green kidneys, this was also true. If so, I can't see what a standardized batch of virus would do at the present time.

DR. MURRAY: Do we have any thoughts from the industrial group on this?

DR. HILLEMAN: I think it would be very handy. I don't know whether you are in the right ball park or not to have a reference standard.

DR. MURRAY: I am sorry, we didn't hear that.

DR. HILLEMAN: I say I think it would be excellent to have such a reference standard for correction purposes, to see whether one is in the right ball park with his titrations and so on. I would welcome it personally.

DR. MURRAY: Well, is the technique of virus growth such that it is possible to get a preparation which would be suitable for this purpose at the present time? And if so, what kind of system should be used for growing the virus? Should the Green monkey tissue culture be used?

DR. MURRAY: Or what titer would be a suitable one to aim for?

DR. MEYER: I think there is a more basic use. Dr. Hilleman expressed the fact he thinks it would be useful. How many people would actually, in other words, if such a preparation was made available, this is a virus material that you can use as a control virus for titer, how many people would actually use this in their tests? The reason I mention it, I remember the early phase of measles investigation, there was a question of putting out such a product, and nobody wanted to use it. I mean it was available and people just didn't want it. It was only when you actually begin to have lots of experimental vaccine made that people had material they wanted to compare the titer on, that they really used it. The investigators working on early measles they had their own titrational standards.

DR. HILLEMAN: With measles you didn't have the problem of this tremendous variation in titers, from test to test. Here I think we would liketo have some frame of reference for what is a reasonable assurance that this is in the same ball park as other people are getting titers. In one laboratory you hear a titer reported as high as 10 to minus 5 or 6 and in another about all you hear is titer minus 3. So it would allow you to check out your test system to make sure it is properly sensitive and would allow one tohave some sort of a guide for what is a reasonable titer and what is not.

DR. BELCOURT: Yes. I agree with Dr. Hilleman, that it is nice to have this type of thing that we can control ourselves with as we are going on and any deviation we run into, we might as well find out early, instead of later on.

DR. BUESCHER: May I ask what this requirement would be? 10 to the 6 ampules of one-tenth cc? Do you use these in recurrent tests?

DR. BELCOURT: It would be nice to have an average titer of say 10 to 5, 10 to 6.

DR. BUESCHER: How many ampules do you want?

DR. BELCOURT: For periodic checking, if we had probably eight or ten ampules, that we could store away and keep, and periodically come back to.

DR. MEYER: If you make such a standard available, you almost have to have at least 5,000 or more ampules of say some 2 cc volume, or 1.5 cc volume. I mean if you don't have at least that many, it is not worth much.

DR. MURRAY: Whether we have it now or whether we defer this until later, I think it is something which will have to be used in connection with vaccine, which is eventually developed. The only question is are things going to change so much within the immediate future that anything we use now is going to have no usefulness by the time we get to the point of considering vaccine for licensing. I think

Dr. Hilleman, by his facial expression, would indicate that, so what, let's use it now.

We could spend time, but I don't think it would be really useful at this time to go into matters of problems of extraneous viruses, in relation to future vaccines. I think people have a better appreciation of the significance of these things now than they had a few years ago. And questions of general safety in man, unless there is some idea that someone has that they think should be brought to the attention of the group.

This of course would also include the question of the system to be used for virus production in the case of potential vaccines.

DR. ROBBINS: I just have one point of view I would like to reiterate, I said it yesterday and I would like to say it again. It seems to me we are in a very different situation with the prospects for rubella vaccine than with measles or polio. And that haste here would be most undesirable. And that if we could free ourselves of the sense of pressure, it would be most helpful. And if we could free ourselves of the kind of pressures that come from the newspapers and magazines and so on, and face the fact that it is unquestionably going to take a number of years before we have a useable and workable vaccine, but this is all right, then perhaps we can save ourselves a few headaches.

DR. MURRAY: I am glad you said that. Does anybody want to make any concluding speeches?

If not, I want to thank you all for coming down here, it has been I think a most profitable meeting, certainly it has been for us and I hope that this feeling is mutual.

We have had some recommendations I think that we can act on. We will get the transcript of the proceedings into a form suitable for transmission to the participants as soon as we can. I can't promise any date on this, however. And I look forward to seeing you at a future date when we have more information to consider.

(Thereupon, at 3:55 p.m. the meeting was concluded.)

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Conference on Rubella, DBS, April 29-30, 1964

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